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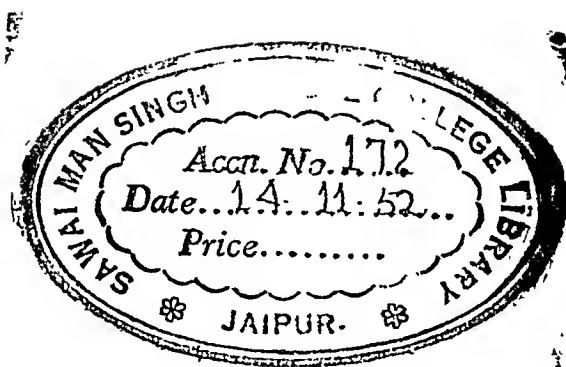
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MEL B IN THE TREATMENT OF HUMAN TRYPANOSOMIASIS^{1,2}

ERNST A. H. FRIEDHEIM

For practical therapeutic purposes, the etiological entity of human Trypanosomiasis presents two entirely different aspects due to the fact that the classical drugs, satisfactory in the first stage of the disease, are inactive in the second stage, and *vice versa*.

In the so-called first stage of human Sleeping Sickness, where the spread of the trypanosomes is limited to blood and lymph glands, hundreds of the approximately 12,000 trypanocidal arsenicals developed since Ehrlich's times and at least a dozen of the metal free urea and amidine derivatives are capable of achieving definite cures.

From this mass of material, a result of 40 years of hit and miss research, three drugs have emerged and are at present in use for the routine mass treatment of the hemato-lymphatic stage by the various African Sleeping Sickness Services: Atoxyl, Bayer 205 (synonyms: Germanin, Moranyl, Naphuride, Antrypol, Suramin B.P.) and Pentamidine.

Of these drugs Pentamidine seems to offer maximum advantages, as it not only cures first stage Sleeping Sickness within 10 days, but also confers a long lasting resistance to re-infection. Unfortunately, all the drugs mentioned so far do not penetrate into the CSF and are therefore of no therapeutic value in the so-called second or meningo-encephalitic stage of Sleeping Sickness. In this stage, only one drug is so far of any help: Tryparsamide. Tryparsamide has two drawbacks: It is unsatisfactory as a trypanocidal agent in the hemato-lymphatic stage of the disease and its usefulness in the second stage becomes more and more curtailed, as the number of tryparsamide fast strains of trypanosomes increases all over Africa.

To give an idea of the size of the problem on hand: In French West Africa, totaling a population of 16 millions, the Sleeping Sickness Service has examined in 1946, 4.6 million persons, detecting over 50,000 new cases of whom 60 per cent were found to be in the second stage of Sleeping Sickness. The Sleeping Sickness Service of the Belgian Congo reports (1947, personal communication) that 80 per cent of all second stage cases detected are tryparsamide resistant.

It follows that the choice of the appropriate drug for the treatment of Sleeping Sickness must be based on a careful differential diagnosis of the stage of the disease, *i.e.* that all cases found to carry trypanosomes in glands and/or blood must be submitted to spinal puncture. The scope of the practical consequences, as to organization and personnel, evolving from this situation, can be appreciated by the fact that the Sleeping Sickness Service of French West Africa performed over 50,000 spinal punctures in the course of the year 1946. It is a further handicap for the organization of mass treatment that two different schemes of treatment have to be established, involving widely different time tables. The

¹ From the laboratory of Ernst A. H. Friedheim, 333 West 52nd St., New York 19, N. Y.

² In collaboration with the Sleeping Sickness Service of French West Africa.

treatment of the first stage with Pentamidine can be completed within 10 days, but the tryparsamide treatment of the second stage, with or without addition of another drug, such as Bayer 205, takes a minimum of 10 weeks, and routinely three such ten weeks courses are applied at three months interval, which brings the duration of the treatment up to one year.

This situation points to the need for a drug which would be curative, by a short course of treatment, indiscriminately in all stages of Sleeping Sickness. This aim seems to have been achieved with p-melaminylphenylarsenoxide (Melarsen Oxide) (1, 3, 4, 5).

Sleeping Sickness of all stages, including advanced second stage cases, have been cured with a Melarsen Oxide treatment consisting of 2 series of 7 intravenous daily injections of 1.5 milligrams per kg. of body weight each, the 2 series being separated by a rest period of one month. These results have been controlled up to 10 months after treatment (2).

Further research in the series of melaminyl arsenicals in this laboratory has led to a new compound designated by "Mel B" which is an alkyl mercapto derivative of melaminylphenylarsenoxide containing trivalent arsenic. "Mel B" is tolerated at significantly higher doses than Melarsen Oxide. This allows doubling the individual dose and shortening of the duration of treatment.

In the following there is described the results of the treatment with "Mel B" in 50 cases of advanced second stage Sleeping Sickness due to *T. gambiense* and transmitted by *G. palpalis*. The treatment was carried out in collaboration with the Sleeping Sickness Service of French West Africa, in previously untreated patients detected in the districts of Bobo-Dioulassou, High Volta, and Gueckedou, French Guinea.

The diagnosis was made by the microscopic detection of trypanosomes in lymph glands (fresh preparation), and/or blood (fresh and stained preparations, triple centrifugation), and by the examination of the CSF, counting the cells in a Fuchs-Rosenthal chamber, determining the protein content, referred to as "albumen," by the Siccard-Cantaloube method, and testing the centrifugate of 10 ml. of CSF for trypanosomes. Normal CSF was routinely defined as presenting not more than 5 cells per mm.³ and not containing more than 0.22 gram of total protein per 100 ml. of CSF.

"Mel B" was used in form of its 5 per cent solution in propylene glycol. The principle of the treatment was based on daily intravenous injections, given in one sequence, or in 2 series separated by a rest period. The total number of injections ranged from 3-14, the total duration of the treatment, including rest periods, from 3-38 days. The single dose ranged from 2.0-4.0 milligram per kg. of body weight. The progress of the patients was controlled by examinations of gland juice, blood and CSF at various intervals up to 222 days after the end of the treatment. Microscopic blood and gland examination were performed at least seven times after the end of the treatment.

IMMEDIATE TRYPANOCIDAL EFFECT

a) *Blood*. Twelve cases presented before treatment trypanosomes in the blood. In all 12 cases the trypanosomes were not to be found by the triple

centrifugation method, 24 hours after a single intravenous dose varying from 1.5–4.0 milligrams per kg. of body weight.

b) *Lymph glands*. All 50 cases treated, presented before treatment trypanosomes in cervical lymph glands. In all 50 cases the trypanosomes were not to be found 24 hours after a single intravenous dose of 1.5–4.0 milligrams per kg. of body weight.

c) *Cerebro spinal fluid*. In 8 cases, presenting before treatment trypanosomes in the CSF, the parasites were not to be found 7 days after a treatment con-

TABLE I

The evolution of cell count and albumen content of the spinal fluid after a short term treatment with Mel B.

NO.	DAYS AFTER TREATMENT	CELLS PER MM. ³	TRYPANOSOMES	ALBUMEN	DOSE
				grams per cent	mg./kg.
49	0	198	0	0.40	3 x 4.0
	15	3	0	0.22	
	53	1	0	0.18	
41	0	196	+	0.40	7 x 2.8
	52	5	0	0.30	
	69	3	0	0.25	
38	0	291	+	0.85	8 x 2.1
	21	36	0	0.50	
	89	5	0	0.40	
192	0	88	+	0.56	4 x 3.6
	9	25	0	0.56	
	33	5	0	0.50	
107	0	48	0	0.40	4 x 3.5
	7	5	0	0.40	
	63	2	0	0.40	
105	0	21	0	0.30	4 x 3.0
	11	5	0	0.25	
	27	5	0	0.50	
	44	2	0	0.22	

sisting of 4 intravenous injections of 3.6 milligrams per kg. of body weight each, given at the rate of 1 a day for 4 consecutive days. In one case, which received only 3 days of treatment with three injections, of 3.6 mgs. per kg. of body weight each, the trypanosomes had disappeared from the CSF 7 days after the treatment.

EFFECT ON THE CSF OF SECOND STAGE SLEEPING SICKNESS

The evolution of cell count and albumen content after a short term treatment. Table I shows the change of the CSF formula as a function of time elapsed since the end of a single course treatment lasting from 3–6 days in 6 cases. It follows, that as few as three injections (case #94), applied in 3 days, may reduce

cell count and albumen content to normal within two weeks following the end of the treatment; but in the majority of cases the general rule that the cell count approaches normalcy more rapidly than the albumen content, applies. In cases where the cell count has returned to normal, the albumen content may continue to remain high for a long time (case #107), or drop more or less slowly (cases #38, 41, 49, 192), occasionally after a temporary raise (case #105), due probably to excessively frequent spinal punctures.

TABLE II

Group I

20 cases of second stage Sleeping Sickness treated with Mel B.

Average control time: 108 days.

Results: Cell count and albumen content of spinal fluid normal.

PATIENT				TREATMENT			CONTROL						
No.	Sex	Age	Weight	Dose	Number of injections	Duration	Cerebro spinal fluid						Duration
							Before			After			
							Cells	Alb.	Trypano-somes	Cells	Alb.	Trypano-somes	
			kg.	mg./kg.		days	per mm. ³	grams per cent		per mm. ³	grams per cent		days
278	M	30	53	3.6-4	8	38	24	0.40	0	2	0.22	0	39
9	F	16	48	2.5-6	9	21	26	0.46	+	3	0.22	0	81
412	F	22	44	2.7	14	21	40	0.56	0	3	0.22	0	168
193	M	6	19	3.6	8	18	76	0.50	0	1	0.22	0	44
32	M	50	50	2.4	10	17	27	0.56	0	2	0.22	0	17
194	F	16	45	3.6	8	15	92	0.56	+	2	0.22	0	109
402	M	14	42	2.1	10	10	17	0.18	0	1	0.20	0	222
26	F	20	41	3.0	10	10	240	0.40	0	2	0.22	0	173
40	M	50	63	1.9	10	10	229	0.30	+	1	0.22	0	180
38	M	30	56	2.1	8	8	291	0.85	+	5	0.22	0	170
41	F	35	43	2.8	7	7	196	0.40	+	1	0.22	0	141
39	F	30	47	2.5	7	7	109	0.30	0	1	0.22	0	178
47	F	10	20	3.6	6	6	107	0.40	0	1	0.22	0	184
198	M	27	50	3.6	4	4	280	0.50	+	2	0.22	0	13
196	M	6	22	3.6	4	4	28	0.50	0	2	0.22	0	12
44	F	32	50	2.4	4	4	74	0.35	0	1	0.18	0	173
105	M	10	31	3.0	4	4	21	0.30	0	2	0.22	0	44
104	M	6	23	3.0	4	4	21	0.40	0	2	0.22	0	46
112	M	8	25	3.6	3	3	30	0.50	+	2	0.22	0	80
49	M	9	15	4.0	3	3	198	0.40	0	1	0.18	0	53

The overall effect on the CSF determined up to 7 months after the treatment. All available data are summarized in tables II-IV. The 50 cases have been classified in three groups: In 20 cases, forming group I, the cell and albumen content had returned to normal at the end of the last control examination, the control period averaging 108 days. In group II, 23 cases are summarized where the cell count, but not the albumen content, had returned to normal after a control period averaging 78 days.

Group III contains 7 cases, where both cell count and albumen content were above normal after a control time averaging 70 days, the cell count being significantly reduced and the albumen content stationary or somewhat reduced.

TABLE III

Group II

23 cases of second stage Sleeping Sickness treated with Mel B.

Average control time: 87 days.

Results: Cell count normal, albumen content of spinal fluid above normal.*

PATIENT				TREATMENT			CONTROL						
No.	Sex	Age	Weight	Dose	Number of injections	Duration	Cerebro spinal fluid						Duration
							Before			After			
							Cells	Alb.	Trypano- somes	Cells	Alb.	Trypano- somes	
			kg.	mg./kg.		days	per mm. ³	grams per cent		per mm. ³	grams per cent		days
28	M	30	58	2.0-3.5	14	84†	340	0.56	+	2	0.56	0	56
114	M	45	67	2.8	8	27	96	0.56	0	2	0.45	0	33
199	F	22	44	3.6	8	25	196	0.56	0	3	0.25	0	18
24	M	32	60	2.0	14	21	234	0.46	+	5	0.40	0	33
399	M	15	35	2.5	14	21	304	0.50	+	5	0.25	0	211
398	F	26	50	2.4	14	21	360	0.56	+	4	0.35	0	205
190	M	57	51	3.6	8	17	112	0.52	0	3	0.45	0	27
401	M	32	65	1.8	10	10	89	0.56	+	3	0.30	0	120
43	M	50	55	2.2	10	10	170	0.65	0	1	0.56	0	170
45	F	30	57	2.1	8	8	56	0.40	0	1	0.25	0	179
42	M	40	59	3.0	7	7	148	0.45	0	2	0.40	0	182
50	M	30	58	2.1	7	7	94	0.45	0	2	0.40	0	15
48	M	11	26	2.3	6	6	120	0.40	0	2	0.25	0	105
113	F	34	42	3.6	4	4	28	0.60	0	8	0.22	0	15
102	M	25	51	3.5	4	4	80	0.56	0	2	0.25	0	33
107	M	13	42	3.5	4	4	48	0.40	0	2	0.40	0	63
197	M	13	33	3.6	4	4	80	0.56	0	2	0.40	0	27
103	M	18	43	4.2	4	4	180	0.71	0	3	0.35	0	12
203	M	16	42	3.6	4	4	16	0.56	0	2	0.30	0	104
106	F	30	45	3.5	4	4	420	0.71	+	3	0.30	0	34
108	F	33	49	3.6	4	4	432	0.75	+	2	0.28	0	88
111	F	22	42	3.6	4	4	120	0.70	0	2	0.30	0	151
204	F	8	28	3.6	4	4	391	0.45	+	1	0.30	0	110

* Vice versa for case No. 113.

† 10 weeks rest period between two courses of injections.

The classification of the cases in the three groups is only temporary. The groups represent stages of recovery through which the patients pass as a function of the time elapsed since the end of the treatment. Obviously, the time required to reach a certain stage of recovery depends to a large extent on individual, uncontrollable factors. As to be expected, the cases showing the least improvement are to be found in group III, corresponding to the shortest average control time,

while the cases returned to normal by all criteria are summed up in group I, corresponding to the maximum average control time. The fact may be emphasized that in none of the 50 cases did control examinations reveal the presence of trypanosomes in the CSF, including 20 cases presenting trypanosomes in the CSF before treatment. Coinciding with the CSF controls, blood and lymph glands were examined, with persistently negative results. It may be stated that after the first injection, no trypanosomes were ever encountered in any body fluid examined.

Clinical amelioration set in coincident with the disappearance of the trypanosomes from all body fluids, and progressed essentially on a parallel with the

TABLE IV

Group III

7 cases of second stage Sleeping Sickness treated with Mel B.

Average control time: 70 days.

Results: Cell count significantly decreased, but above normal. Albumen content of spinal fluid stationary or decreased, but above normal.

PATIENT				TREATMENT			CONTROL						
No.	Sex	Age	Weight	Dose	Number of injections	Duration	Cerebro spinal fluid						Duration
							Before			After			
							Cells	Alb.	Trypano-somes	Cells	Alb.	Trypano-somes	
			kg.	mg./kg.		days	per mm. ³	grams per cent		per mm. ³	grams per cent		days
411	F	40	40	2.2	14	21	340	0.50	0	7	0.25	0	166
25	M	46	58	2.0	14	21	812	0.50	+	48	0.50	0	33
403	M	25	61	2.0	10	10	184	0.56	+	16	0.40	0	33
200	F	26	44	3.6	4	4	380	0.40	+	6	0.40	0	14
277	F	25	62	4.0	4	4	125	0.90	+	12	0.60	0	37
202	M	10	27	3.6	4	4	80	0.56	0	7	0.40	0	125
192	M	32	63	3.6	4	4	88	0.56	+	8	0.40	0	83

decrease of the cell count, but largely independent from the albumen content of the CSF.

Comatous stretcher cases usually started to rise and walk without help one week after the first injection.

Further controls will have to bring out the significance of the above-normal albumen content of the CSF persisting in a number of cases at the end of the observation period. High total protein content, in the absence of other symptoms, may signify nothing but a clinically meaningless residual "scar" effect or a precursor of a relapse. Incidentally, this question of "residual" albumen content of the CSF calls urgently for further research. Thousands of former patients all over Africa continue to be treated with large amounts of arsenicals as a precautionary measure, although they are clinically cured and present no other pathological change than a high albumen content. It is suggested that

a qualitative analysis of the CSF albumen may help to select the cases where further treatment is really required.

TOLERANCE

Within the dose limits indicated the treatment with "Mel B" had no untoward effects. Symptoms of intolerance sought included albuminuria, gastrointestinal disturbances, skin manifestations, nervous and in particular, visual disorders. In 15 cases chosen at random, the white blood formula was checked and found to be unaffected.

SUGGESTED COURSE FOR THE TREATMENT WITH MEL B

On the basis of the experience gained to-date, the following routine is suggested for the mass treatment of Sleeping Sickness in all stages: Two series of 4 intravenous doses of 3.6 milligrams/kg. each, applied one dose a day for 4 consecutive days, the two series being separated by a rest period of one week.

The scheme should not be applied indiscriminately to cases with extreme symptoms of meningo-encephalitis, such as semi-coma and coma. In such cases the dosage indicated may be retained, but an interval of several days should be allowed between the first 3 or 4 injections, in order to avoid possible severe Herxheimer type reactions due to sudden massive destruction of parasites in the nervous centers.

The question remains open whether it is necessary to apply the second series of 4 injections to first stage cases. Preliminary data point in this direction, but until definite results are available the full course of 8 injections is recommended.

SUMMARY

1) "Mel B," a new trivalent arsenical has been applied to the treatment of 50 cases of human second stage *T. gambiense* Sleeping Sickness, of which 20 cases presented trypanosomes in the CSF.

2) The cases have been controlled up to 7 months after the treatment.

3) The treatment had no untoward effect.

4) The trypanosomes disappeared in all cases without reoccurrence from lymph glands and blood, 24 hours after the first injection.

5) "Mel B" penetrates into the CSF and exerts a strong parasitocidal effect on the trypanosomes in the central nervous system.

6) A single series of 3-4 daily injections of "Mel B", i.e. a treatment of 3-4 days duration, may restore the pathological CSF changes of advanced meningo-encephalitis Sleeping Sickness cases to normal co-incidental with clinical recovery.

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THE ACTIVE FORM OF TRYPARSAMIDE, TPB, IN THE TREATMENT OF HUMAN TRYPANOSOMIASIS^{1,2}

ERNST A. H. FRIEDHEIM, M.D., PH.D.

The pentavalent aromatic arsenicals are endowed with trypanocidal activity only in as far as they are reduced in the organism to their trivalent derivatives. It is the task of chemotherapy to relieve the organism of the reduction work by performing it in vitro. In other words, the trivalent state is the form of choice in which arsenicals have the best chance to be effective remedies against trypanosomiasis.

These fundamental concepts, developed by Ehrlich, have been applied by Warrington Yorke (1) and his school to the special case of tryparsamide.

In vitro, the trypanocidal activity of tryparsamide is insignificant, the minimal trypanocidal concentration being 1:1600. The reduction to the corresponding arsenoxide increases the trypanocidal activity by a factor of 125,000, corresponding to a minimal trypanocidal concentration of 1:200,000,000. Following an intravenous injection, tryparsamide is rapidly excreted. According to Launoy and Fleury (2), from 88-95 per cent is eliminated by way of the kidneys within the first hour after the injection. The dose of tryparsamide injected into a patient has a therapeutic effect only in-so-far as the small percentage of the pentavalent drug retained by the organism is reduced to the trivalent form. Thus, every treatment loads the organism with a considerable quantity of drug, which is not operative, presenting nothing but dead weight.

The logical consequence of this situation would postulate reduced tryparsamide for the treatment of Sleeping Sickness. The Liverpool group has taken this step, using the reaction product of thioglycolic acid and tryparsamide. According to expectations this combination was found to possess a high direct trypanocidal activity, but unfortunately this advantage was counterbalanced by a prohibitive toxicity, and chemical instability.

These difficulties have now been overcome by a method of stabilisation and detoxification of reduced tryparsamide, developed in this laboratory and to be described elsewhere. Preliminary tests, to be described in the following, tend to demonstrate that the new product, designated by "TPB", represents truly the "active" form of tryparsamide, which combines both high "peripheral" trypanocidal activity and the propensity to clear up readily trypanosome meningo-encephalitis.

TPB is a white powder, containing 21.6 per cent trivalent arsenic.

Treatment with TPB is carried out in daily doses, either by mouth or by intravenous injection of a solution in an appropriate organic solvent.

For the time being, the routine dosage has been fixed as follows:

By mouth: Single dose, 0.010 gm./kg. One dose per day for 8 consecutive days. This series may be repeated after an interval of one week.

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² In collaboration with the Sleeping Sickness Service of French West Africa.

Intravenous injection: Single dose, 0.0015 gm./kg. One dose per day for 5 consecutive days. This series may be repeated after an interval of one week.

The treatment with TPB has been applied to 10 previously untreated cases of second stage Sleeping Sickness of which two presented trypanosomes in the CSF.

The diagnosis was made by microscopic examination of gland juice, wet and stained preparations of fresh blood and of the centrifugate (triple centrifugation) of 10 ml. of citrated blood. All cases were submitted to spinal puncture. The CSF was examined as to cell count, total protein content as determined by the Siccard-Cantaloube method, and presence of trypanosomes in the centrifugate of 10 ml. of CSF.

Within the dose limits indicated, the treatment did not give rise to any untoward effect. Symptoms of intolerance sought for included gastro-intestinal, cutaneous, nervous and particularly optic, disturbances. The white blood formula was checked in all cases.

TABLE I

Immediate trypanocidal effect of a single intravenous injection of 1.5 mg./kg. of TPB on lymph gland trypanosomes

	HOURS ELAPSED BETWEEN TREATMENT AND CONTROL OF GLAND JUICE				
	$\frac{1}{2}$	1	3	6	24
Number of patients examined.....	1	1	2	2	5
Number of patients negative.....	0	1	2	2	5

IMMEDIATE TRYPANOCIDAL EFFECT OF TPB IN THE BLOOD AND LYMPH GLAND INFESTATION

Treatment by mouth. In 5 cases, presenting before treatment trypanosomes in the gland juice, no trypanosomes were to be found in the glands 24 hours after a single oral dose of 0.007–0.025 gm./kg. In one case, presenting trypanosomes in glands and blood, the blood became negative 12 hours after a single oral dose of 0.005 gm./kg. while the gland juice remained positive. The trypanosomes disappeared from the glands 36 hours after the onset of the treatment with a total dose of 0.015 gm./kg.

Treatment by intravenous injection. Five cases presenting trypanosomes in the lymph glands, and of whom 2 cases also presented trypanosomes in the blood, were treated with a single intravenous injection of 1.5 gr./kg. of TPB. Gland juice and blood were examined at various intervals. The results are summarized in Table I.

It follows that $\frac{1}{2}$ hour after the treatment the gland juice was still positive, while examinations carried out one hour or later after the treatment, were negative.

In the two blood positive cases, examination of wet preparations, stained thick drops of the fresh blood and the centrifugate of triple centrifugation, showed

that the trypanosomes had disappeared, one and 3 hours respectively, after the treatment.

The microscopic examination of glands and blood was repeated daily for 3 days and confirmed that the trypanosomes had disappeared in the aftermath of a single injection of TPB.

EFFECT OF ORAL TREATMENT WITH TPB ON THE CSF CHANGES OF SECOND STAGE SLEEPING SICKNESS

The effect of early treatment with TPB on the pathological changes of the CSF of 5 cases of second stage Sleeping Sickness is summarized in Table II.

TABLE II
Effect of oral treatment with TPB on CSF of second stage Sleeping Sickness

PATIENT NO.	TREATMENT			CONTROL						
				Cerebro spinal fluid						Duration*
	Single dose	Num-ber of doses	Duration of treat-ment	Before			After			
				Cells	Alb.	Tryp.	Cells	Alb.	Tryp.	
	mg./kg.		days	per mm. ³	gr. %		per mm. ³	gr. %		days
L 13	7	7	7	29	0.30	0	4	0.22	0	7
299	8	10	19	256	1.11	+	10	0.40	0	53
79	10	14	21	24	0.50	0	1	0.22	0	162
80	25	8	11	32	0.56	0	2	0.22	0	161
414	11									
	First series	8	8	454	0.46	+	43	0.30	0	5
	Rest period		7							After 1st series
	Second series	8	8				7	0.30	0	8
		—	—							After 2nd series
	Total	16	23							

* Time elapsed between last treatment and control spinal puncture.

It follows that TPB absorbed from the gastro-intestinal tract rapidly reaches and destroys the trypanosomes in the central nervous system and turns cell count and albumen content of the CSF towards normalcy. Case No. 414 (Table II) who was treated with 2 series of 8 daily oral doses of TPB, is particularly demonstrative in this respect.

In two cases the return of the CSF to normal was verified 5½ months after oral treatments lasting no more than 11 and 21 days respectively.

THE EFFECT OF TPB ON TRYPARSAMIDE RESISTANT TRYPANOSOMES

TPB was tested in one human second stage case, and in one guinea pig infected by a bite of glossinae infected with tryparsamide resistant strains of *T. gambiense*. This study was carried out at the Institute for Tropical Medicine

at Leopoldville, Belgian Congo. TPB was without effect on the CSF changes of the patient and only cleared the trypanosomes from the blood of the guinea pig temporarily.

This confirms the observations of the Liverpool School to the effect that resistance against pentavalent tryparsamide coincides with resistance against trivalent reduced tryparsamide.

This leaves for the present the arsenicals derived from *s*-triazine, *i.e.* Melarsen (3), Melarsen oxide (4, 6, 7), and Mel B (5) as the only chemotherapeutic agents capable of ameliorating the condition of the CSF of tryparsamide resistant second stage Sleeping Sickness. Nevertheless, trivalent tryparsamide in the form of TPB may be useful in regions free from tryparsamide resistant trypanosomes, where it will allow treatment of all stages of Sleeping Sickness with a single drug and within a time limit, significantly shorter than the duration of a typical tryparsamide treatment.

SUMMARY

TPB represents the "active form", *i.e.* the arsenoxide of tryparsamide in a stabilized and detoxified form, combining high trypanocidal activity towards the trypanosomes in blood and lymph and CSF, with the propensity to clear up readily the trypanosome meningo-encephalitis.

TPB is without effect on tryparsamide resistant trypanosomes.

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A FIVE DAY PERORAL TREATMENT OF YAWS WITH STB, A NEW TRIVALENT ARSENICAL¹

ERNST A. H. FRIEDHEIM

The treatment of yaws, in individual cases, does not present any particular problem. Penicillin, arsenicals such as arsphenamine, mepharsen, acetarsone, carbarsone and any one of the numerous bismuth preparations may achieve a cure.

The problems arise in connection with mass treatment in the tropics, particularly in the West African bush, where the incidence of yaws reaches 75 per cent and hundreds of thousands of yaws patients are scattered in innumerable small villages over huge territories.

A chemotherapeutic agent, to be useful under such conditions, must possess the following characteristics:

1. *Wide margin of safety.* The tolerance of African populations for arsenic and antimony preparations varies widely from one territory to another, due to racial, nutritional and climactic factors. A scheme of tryparsamide treatment, perfectly tolerated in Nigeria, gave rise to a death rate of 5 per cent in Western Sierra Leone. Tartar emetic has had to be banned altogether from use in the Kissi tribe of Sierra Leone, where even greatly reduced doses caused death.

2. *Ease of administration.* The treatment has to be performed by native personnel with no or only limited medical training, who daily have to handle large numbers (hundreds) of patients. This precludes intravenous injections.

3. *Short course of treatment.* Uneducated Africans will follow a treatment only until the worst symptoms have disappeared. A scheme prescribing 10 weekly bismuth injections will be followed by only a minority of patients beyond the third or fourth injection.

4. *Stability under tropical conditions.* The temperature in a routine metal medicine chest, carried as head load in the sun, has been found to reach 75°C.

5. *Low cost.* The limited budgets of many colonial health agencies only will allow for intensive yaws campaigns by procedures in which the expenses for drugs, personnel and equipment are at a minimum.

These practical considerations rule out the trivalent arsenicals arsphenamine and mepharsen, and restrict the choice to acetarsone and bismuth preparations, both of which may be applied by intramuscular injections. What is at present one of the most practical and effective methods of putting arsenic and bismuth to use, is the scheme of the Sierra Leone Health Service, prescribing three pairs of simultaneous weekly injections of acetarsone (2.5 cc. of a 23.6 per cent solution) and bismuth subsalicylate (2 cc. of a 10 per cent oil suspension). The treatment is thus completed in 3 weeks.

It would appear that peroral application should present the optimum method

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for mass treatment under the conditions contemplated. Acetarsonsone and Carbarsonsone are the only preparations recommended for the oral treatment of yaws. Neither drug has been approved by an official African health authority for systematic mass treatment of yaws.

Forty years ago Paul Ehrlich pointed out that the relatively inactive pentavalent arsenicals are reduced in the organism to an active trivalent form, and that chemotherapy therefore preferably should utilize trivalent arsenicals.

So far this principle has not been applied to the case of acetarsonsone.

We have now prepared a relatively non-toxic and stable form of 4-oxy-3-acetylaminophenylarsenoxide, *i.e.* the trivalent derivative of acetarsonsone, and find that this compound, designated by "STB", approaches closely the characteristics set forth as being desirable for mass treatment in undeveloped territories.

The clinical tests have been carried out in the mountainous forest regions of French Guinea, in the district of Gueckedou, where luxuriant vegetation, ample rain fall, and a population of low social and economic order provides an ideal ground for yaws. A survey of 60 villages in the canton of Guende, totaling a population of 5000, revealed an overall incidence of yaws of 5 per cent and in individual villages an incidence as high as 17 per cent.

Eighty eight cases presenting definite classical yaws lesions, 73 of the infectious, 15 of the non-infectious type, were chosen for the study. The infectious cases include primary and secondary lesions, typical isolated or multiple framboesomata, condylomata and granulomatous ulcers of the soft skin and open plantar lesions. The non-infectious cases are represented by covert palmar and plantar lesions (crab yaws).

The treatment consisted of a single oral daily dose of 0.01–0.02 gr./kg. on 5 consecutive days.

The treatment in the doses indicated did not give rise to any signs of intolerance. Symptoms looked out for included: thermal reactions, skin eruptions, albuminuria, anorexia, stomatitis, vomiting, pains, diarrhea. Blood counts taken at random in 20 cases revealed no pathological changes.

Clinical control of the cases treated was performed 3 months after the treatment.

THERAPEUTIC RESULTS

On the second to third day of treatment all exudative lesions were dry and covered by crusts. On the fifth and last day of treatment all previously open lesions were covered by epithelium to which crusts were adherent in some cases, while in others the scabs had dropped. At this time the scars of lesions which had been thickly infiltrated, were still slightly elevated. At the end of the treatment the foot soles had become painless and non-sensitive to pressure in 40 out of 48 crab yaws patients. Crevasses and fissures were healed.

In 8 crab patients pains had diminished, but the plantar region was still sensitive to pressure and some fissures and ulcers persisted.

Three months after the treatment 87 of the 88 cases of this group were cured. The cures include all of the 73 infectious cases. The single "failure" concerns

one case of plantar yaws, where typical plantar yaws lesions were accompanied by perforating ulcers and interdigital fissures with mixed infections. At the time of the control the typical lesions were healed while the atypical ulcers and fissures persisted. As it is not possible to evaluate the relative role of yaws and complicating factors, this case is classified as failure.

For the purpose of this survey "cures" are defined as follows:

Infectious forms. The yaws lesions have disappeared. Their place is taken by unbroken skin which may show pigment abnormalities: Hyper- and hypopigmentation, each alone or both in various combinations, such as hyperpigmented center and hypopigmented periphery or vice versa. In rare cases several zones of hyper- and hypopigmentation alternate. The epidermis of ancient lesions may be slightly atrophic, tissue paper like, or present a certain degree of hyperkeratosis. The scars are usually quite flat, but may present some minor degree of thickening.

Plantar yaws. Painful fissures and depressed areas with partly decomposed horn layer and fungus-like protrusions surrounded by circular fissures have disappeared. The site of past lesions is usually clearly outlined, as the newly formed hornlayer has not yet reached the level of the surrounding plantar surface. Function, previously impaired by pain, is restored as the patients regain the use of their feet, walking on stony trails and climbing rugous trees.

Palmar yaws. Four such cases have been treated. Before treatment the palms were swollen, taut, painful, covered by polymorph map-like zones of desquamation, precluding manual labor. Two months after treatment all pathological changes had disappeared and normal function was restored.

COMMENT

The fact must be emphasized that the control period, limited to three months, is too short to permit final conclusions as to the stability of the therapeutic effect observed. Nevertheless, it may be stated that the simple and rapid treatment described has rendered one hundred per cent of the infectious cases non-infectious, thereby restricting significantly the source of further contamination. This result would seem to justify this report and further clinical investigations.²

The duration of the treatment, viz. 5 days, is no definitive or essential feature. Tests with increased dosage are under way with a view to abbreviate the treatment.

² A second series of 75 cases, 71 of infectious (primary 4 and secondary 67 cases) and 4 of non-infectious tertiary yaws was treated in the Belgian Congo at Kangu in collaboration with Dr. M. Kivits according to the same pattern, with one daily dose of 0.01 to 0.02 gr./kg. of "STB" for 5 consecutive days with results confirming in all respects the observations made in French Guinea.

According to a report received from Dr. M. Kivits, all cases were found to be clinically cured one month after the treatment, except one case of gangosa, which remained unchanged. Five months after the treatment 69 out of 75 cases (92 per cent) were clinically cured, while 5 cases (7 per cent) had relapsed. 1 case showed after 5 months a psoriasiform skin lesion of uncertain etiology.

SUMMARY

1. STB, a stabilized form of 4-oxy-3-acetylamino-phenylarsenoxide has been applied in a five day peroral treatment to 88 cases of yaws and brought about cicatrization of 73 out of 73 cases of infectious yaws and 14 out of 15 cases of "crab yaws."

2. No untoward effects occurred.

3. The five day oral treatment of yaws described seems to be appropriate for mass treatment in tropical undeveloped countries.

THE VIABILITY OF *E. HISTOLYTICA* CYSTS IN SOIL¹

PAUL C. BEAVER AND GUILLERMO DESCHAMPS²

Irrigation with polluted water, use of nightsoil, pollution by flooding, or direct deposition of human feces may carry cysts of *E. histolytica* onto vegetable gardens and truck farms. Seepage and tilling of the soil would tend to separate the cysts from the fecal mass and distribute a portion of them below the surface where they would be protected against direct sunlight, high temperature, and desiccation. Under these conditions, providing the soil itself is not a hostile environment, cysts might remain viable for relatively long periods, be transported to the markets, and thus become a potential source of infection. It has been assumed that this does occur. Craig (1) states that uncooked vegetables from gardens fertilized with nightsoil are a very common source of infection in those countries where the practice prevails; and Andrews' study (2) of an unsanitated mining community in Mexico indicated that the high incidence of amebic infection found there probably was due to the use of raw sewage for irrigating the vegetable gardens. However, direct observations have not been made on the survival of *E. histolytica* cysts in soil.

It is well known that cysts of *E. histolytica* survive for several days or even weeks in water, but perish in a relatively short time in feces, survival in each instance being dependent primarily upon temperature. At high summer temperatures cysts will survive not more than a day or two in feces, whereas they generally survive much longer periods in clean well-aerated water (1). The present study indicates that they may survive even longer periods in certain types of soil.

MATERIALS AND METHODS

Cysts were obtained from stools of known carriers. They were washed from the feces by repeated centrifugation, using only distilled water. The clean sediment was rinsed onto damp soil and worked into the upper 10–15 mm. of the surface layer. The samples of soil were prepared several days in advance of inoculation. Glass cylinders 15 cm. x 15 cm. were placed in shallow bowls and covered with saucers. A 3–4 cm. layer of coarse gravel was placed in the bottom to insure good drainage and covered with 8–10 cm. of the soil which, after dampening and being allowed to stand for 3–4 days, had the appearance, odor, and texture of well-tilled garden soil. No attempt was made to sterilize the soil. The type

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of soil used most successfully was dark loam containing perhaps 30-50 per cent of fine sand. This type was selected as being most easily maintained in a damp condition without destroying its open granular structure. As a control, cysts were concentrated by zinc sulfate flotation, washed thoroughly to remove the zinc sulfate, and held in cotton-stoppered 125 cc. Erlenmeyer flasks. To insure good aeration, the water was kept at 10 mm. or less in depth and was changed every 24 to 48 hours. Only distilled water was used.

At 2-day intervals the water control and the soil were sampled and tested for the presence of viable (culturable) cysts. The sample from water was merely washed by centrifugation and the sediment put into Balamuth's culture medium (3). The soil sample was suspended in distilled water, strained through four thicknesses of surgical gauze, washed until the supernate was clear, concentrated by zinc sulfate flotation, rewashed and the sediment put into Balamuth's medium. Cultures were examined at 24 and 48 hours. Negative cultures at 24 hours were transferred to 2 tubes of fresh culture medium and read at 24 and 48 hours.

The study was carried out in the Creole Petroleum Company Hospital at Caripito, Monagas, Venezuela in late June and early July, 1947, during the rainy season. All tests were run at room temperature which was between 28° and 34° C.

OBSERVATIONS

Altogether 11 sets of observations were made. The first 5 of the series were pilot tests in which diverse materials and methods were employed. It was shown by these preliminary experiments that the cysts of *E. histolytica* could be recovered from soil by zinc sulfate flotation, that under favorable conditions they would remain viable in soil for at least 2 days, and that free-living species of amebae could not be cultured from the soil in Balamuth's medium at 37.5° C.

The following three experiments, carried out for 6 to 8 days each, provided more definitive information.

Experiment I. Cysts used in this experiment were all mature, having no chromatoidals, no observable glycogen, and 4 nuclei each. The stool was formed. It had stood for 4 hours at room temperature and 4 hours in the refrigerator at about 14° C. Positive cultures were obtained from the soil after 2, 4, and 8 (but not 10) days, and from water after 2 days only. Trophozoites were abundant in all positive cultures.

Experiments II and III. Only immature cysts with one or 2 nuclei were observed in the mushy-diarrheic stools used for these experiments. Many of the cysts had conspicuous chromatoidals. In experiment II, the stool had stood about one hour at room temperature and 12 hours in the refrigerator before it was washed in preparation for the tests; the stool used in experiment III had stood less than 2 hours when used. Results were similar in the two experiments. Positive cultures were obtained from soil on the 2nd, 4th and 6th days, whereas cultures from the controls in water were positive on the 4th but negative on the 6th day in both experiments. Numerous vigorous, actively motile trophozoites were observed in all positive cultures. Presumably the soil still contained culturable cysts after the 6th day when the work had to be discontinued.

DISCUSSION

Some of the essentially negative results in 8 of the 11 experiments (including 5 pilot tests) are readily explained. In three instances cysts were not culturable when washed directly from the newly procured stools which were said to be only 2-6 hours old. These stools may have been older than they were represented to be. Also, two of the soil types used were apparently unfavorable for amebic cyst survival. A sample containing a high proportion of coarse sand tended to be either dry or wet instead of damp, and a clayey sample became very sticky and dense when dampened by adding cysts in water. Finally there can be but slight doubt that the failure of cysts to survive in feces, water, and soil was due largely to the high prevailing temperatures. Since washed cysts in clean water previously have been found to survive less than 10 days at room temperatures (1), it is to be expected that at high summer temperatures, ranging up to 34° C., they would survive a much shorter period.

CONCLUSIONS

Under the conditions of these experiments, cysts of *E. histolytica* have been shown to live at least 8 days in soil, whereas in clean aerated (shallow) water they are culturable for 4 days or less.

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THE EFFECT OF ACETIC ACID ON THE VIABILITY OF *ENDAMOEBIA HISTOLYTICA* CYSTS¹

PAUL C. BEAVER AND GUILLERMO DESCHAMPS²

Foreign residents in tropical or other poorly sanitated regions are faced with the problem of protecting themselves against food-borne enteric infections, and at the same time of satisfying the need and desire for balancing their diets with uncooked fruits and vegetables. As a protection against amebic infection it has been recommended that uncooked foods be dried, dipped in boiling water, or treated with certain chemicals (1). While these methods may be effective from the standpoint of prophylaxis, they are undesirable from the standpoint of appearance and flavor of the foods, and in some instances the chemicals themselves may be hazardous to health. Consequently it is sometimes suggested that all suspicious foods be avoided in the tropics (2, 3, 4). In many cases, however, the foreign resident in the tropics may choose to live in fear of enteric diseases rather than deny himself fresh fruits and vegetables.

The present study suggested itself while we were investigating another problem in a community where fresh fruits and vegetables were of very high quality and were abundant; and where a foreign population was living more or less contiguously with a comparatively much larger native population in which amebiasis was highly endemic and from which were drawn the domestic servants and food handlers for the market, club, hospital, and residences. Since these native employees were in constant contact with the general population, regardless of the vigor with which the food handlers were checked and treated, there were always many carriers among them. Assuming that under these conditions a significant amount of the amebiasis in the foreign group was being spread via uncooked foods, prophylactic measures other than, or in addition to, those directed at the food handlers seemed to be worthy of investigation.

The ideal prophylactic chemical would be lethal to *E. histolytica* cysts in a short period of time, non-deleterious to health in the concentrations used, relatively innocuous from the standpoint of appearance and flavor of at least the majority of the choice fresh vegetables, readily available, and inexpensive. We have obtained experimental evidence which indicates that acetic acid in the concentrations generally used in vinegar (around 5 per cent) comes close to fulfilling

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the requirements of the ideal disinfectant. Apparently acetic acid has not previously been studied as an amebic cysticidal agent. As the principal active constituent of natural vinegars, it is a well-known food preservative; and its toxicity for bacteria, yeasts, and molds has been the subject of investigations which have demonstrated that, at room temperature, 15 minutes contact (or less) with 5 per cent concentration (or less) kills several strains of food poisoning staphylococci, *Salmonella typhi*, *S. aertrycke*, *Escherichia coli*, and others (5). It has further been shown that acetic is more actively germicidal than other common organic acids and that it destroys organisms at a higher pH value than does hydrochloric acid (6, 7, 8).

MATERIALS AND METHODS

Most of the experiments were conducted in the hospital laboratory of the International Petroleum Company, Talara, Peru, July and August, 1947. Additional observations were made in our own laboratories in March and April, 1948. Known carriers of *E. histolytica* brought stools to the laboratory immediately upon passage. Specimens rich in mature cysts were washed by repeated centrifugation and the washed sediment was distributed equally in a series of bacteriological test tubes of the same size (5" x $\frac{5}{8}$ ") as was routinely used for ameba cultures. In most cases the amount of washed sediment in each tube was around 0.02 cc. No attempt was made to estimate the number of cysts in the sediment but they were relatively abundant in all of the material used. Series were set up to test both time and concentration of acetic acid necessary to kill the cysts. Viability of cysts after treatment was determined by culturing in Balamuth's all-liquid medium (9). At least 2 tubes were used as controls in which the cysts were untreated beyond washing. All washing was done with distilled water.

In the test series the acid was added so as to almost fill the tube. Mixing was accomplished by rotating and shaking the tube at 2 to 5 minute intervals. At the end of the test period the tubes were centrifuged, the acid was poured off, the sediment washed 5 times to remove the acid, and the culture medium added to the sediment. Cultures were examined after 36-48 hours and were reported as negative if no amebae could be found by systematic search of one entire drop of carefully selected sediment.

The techniques employed were in some respects unsatisfactory. The contact period for each of the test samples could not be exactly determined because the time necessary to centrifuge, pour off, and refill 8-16 tubes was somewhat variable. Our "5 minute" exposures were actually sometimes a bit less and on occasion perhaps as much as an entire minute more. The least satisfactory of our procedures was the method of keeping the cysts suspended. There was some variation in the amount of flocculation and clump formation, and it was impractical to give each tube and each test series exactly the same amount of agitation.

RESULTS

In general, cultures were either negative or contained many active trophozoites. All negative cultures at critical levels were transferred and two transfer

cultures of the 2.5 per cent acid series proved to be positive. Controls in all of the experiments contained an abundance of active amebae.

Ten per cent acetic acid killed the cysts in a very short time. Three separate tests demonstrated that all cysts were dead in less than 5 minutes. This concentration of the acid can not be used on vegetables, however, because it wilts them quickly. Furthermore, very few natural vinegars contain as much as 10 per cent acetic acid, and when they do they are palatable only after dilution.

Two and one-half per cent strength also killed the cysts but the time required makes it impractical for prophylactic purposes in most instances. Positive cultures were obtained after immersion up to 45 minutes. Negative cultures were obtained after one 50-minute and two 60-minute exposures. Only dead cysts were observed in a culture after 40 minutes' exposure but the transfer was positive.

TABLE 1

Results from culturing washed cysts of E. histolytica after various periods of contact with 5% acetic acid

MINUTES CONTACT WITH 5% ACETIC ACID	EXPERIMENT								TOTAL	
	I	II	III	IV	V	VI	VII	VIII		
	Cyst source								Posi- tive	Nega- tive
	MR	MR	MR	MR	LA	LE	RO	WA		
5		+(1)			+(2)	+(3), -(1)	+(1), -(3)	+(2)	9	4
10	-(1)*	+(1)	-(4)	-(5)	-(2)	-(4)	-(4)	+(1), -(1)	2	21
15		-(1)	-(4)	-(5)	-(2)	-(4)	-(4)	-(2)	0	22
20	-(1)	-(1)	-(2)	-(3)					0	7
30	-(1)								0	1
Control	+(3)	+(2)	+(2)	+(4)	+(3)	+(2)	+(4)	+(2)	22	0

* Numerals indicate the number of individual lots of cysts exposed and cultured at each time interval. For example, in experiment VI, 3 out of 4 tubes of washed cysts were positive after 5 minutes exposure.

Five per cent strength killed the amebic cysts in 10 to 15 minutes (Table 1). In 2 out of 8 series (2 of 21 test lots), positive cultures were obtained following exposures of 10 minutes duration. These cultures contained, along with active trophozoites, many dead cysts. In one of our preliminary tests positive cultures were obtained from 15- and 45-minute tests. However, as was generally true in cases of negative cultures, dead cysts (opaque, granular cytoplasm withdrawn from the cyst wall) were observed in large numbers in the cultures and in view of results obtained from the other test series, it is almost a certainty that the trophozoites were present as a result of faulty technic. Special precautions against contamination resulted in negative cultures from 22 individual 15-minute tests in subsequent experiments. Actually, only 5 minutes exposure frequently killed all cysts, i.e., resulted in negative cultures. Four of the 15 lots given 5-minute exposures were negative. Controls consistently gave positive cultures.

Acetic acid in 5 per cent strength was tested on tomatoes, carrots, radishes,

green onions, celery, cabbage, and lettuce. Color change occurred in radishes only. After 15 minutes there was a permanent change in the flavor of cabbage, carrots, and radishes but it was characteristic of these particular vegetables in salads and would not be regarded as unpleasant to most tastes. After 15 minutes' treatment, radishes were slightly wilted; after 30 minutes they were definitely wilted as was also the lettuce. Cabbage wilted appreciably after 30 minutes.

Vinegar of two different varieties was available for tests in Talara. The better type is made from grapes and is called *vinagre de vino*. The so-called *chicha* is produced by fermentation of grain and contains approximately 2 per cent acetic acid and alcohol in about the same concentration. One sample only of each type of vinegar was tested. Full strength *chicha* did not affect the culturability of cysts in 1.5 hours. Although by rough titration against sodium carbonate the wine vinegar's strength was determined to be around 6 per cent, its action apparently was not equivalent to 5 per cent acetic acid. At full strength it failed twice in 4 trials to kill in 15 minutes and on one occasion (out of 2 trials) some cysts survived 20 minutes' exposure. In the latter case, however, the tube was shaken only once during the 20 minute interval. Definite conclusions regarding the relative value of vinegar as compared with acetic acid in equal strength must await further observations.

In the same manner as the 5 per cent acid and the vinegars were tested, a salad dressing mixture consisting of approximately 5 per cent acetic acid, 20 per cent (by volume) cane sugar, and 1.7 per cent (by volume) salt was made up according to cookbook methods and tested. In the first series, some of the cysts survived 5-, 10-, and 15-minute exposures. In a second series of tests on a newly mixed batch, cultures were negative following exposures of 10, 15, and 20 minutes. Untreated controls were strongly positive in both series.

Citric acid in strengths up to 20 per cent for one hour did not injure the cysts. On the contrary, treatment with citric acid and with N/2 hydrochloric acid enhanced the culturability of *E. histolytica* cysts. Growth in the test cultures was in almost every case more vigorous than in the controls. Increased culturability of *E. histolytica* cysts following treatment with hydrochloric acid has already been observed and interpreted as being due to destruction of bacteria rather than to direct action upon the amebic cyst (10). Oftentimes it appeared that, in addition to the amebae, our cultures contained only one or two species of bacteria—usually long, spore-forming rods which tended to form a pellicle at the surface.

DISCUSSION

The amount of enteric infection to be avoided by sterilization of all fresh fruits and vegetables is unpredictable. Therefore, it could not confidently be stated that using acetic acid treatment necessarily would be effective as a prophylactic measure. However, where conditions are such as to urge the use of all possible preventive measures, it certainly should be borne in mind that acetic acid will kill both amebic cysts and at least certain ones of the pathogenic bacteria in a dilution which is commonly used to improve the flavor of certain foods and is

not particularly objectionable from the standpoint of flavor and appearance of many others; and that there is no other more effective substance known that can be safely added to foods. Therefore, at the present time if chemical treatment of foods is to be used at all, acetic acid would seem to be the chemical of choice.

CONCLUSIONS

1. Continuous immersion in 5 per cent acetic acid has been found to kill the fresh cysts of *E. histolytica* in ten to fifteen minutes.
2. Up to thirty minutes immersion in 5 per cent acetic acid does not adversely affect the flavor and appearance of most fresh vegetables.
3. Citric acid in strengths up to 20 per cent (and N/2 hydrochloric acid) do not kill amebic cysts within an hour.

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A NOTE ON THE APPARENT ANTAGONISM OF A BACTERIAL INTOXICATION TO A PLASMODIAL INFECTION¹

MARK F. BOYD² AND RAFAEL D. COELHO

Without raising the question of the *modus operandi* of malariatherapy in syphilitic infections, instances of antagonism between microbial infectious agents are not so frequent as to be commonplace, while the expanding attention currently paid to substances collectively characterized as *antibiotics*, heightens general interest in such relationships. This consideration prompts the report of the experience of a patient on the Malariatherapy Service of the Florida State Hospital, the course of whose illness is tentatively regarded as affording an instance of such antagonism.

Patient L. M., a badly disoriented, uncooperative and disorderly colored male aged 35 years, was committed to the Florida State Hospital on April 11, 1946. Physical examination subsequent to admission was substantially negative in all respects except insofar as it related to the central nervous system. In view of his subsequent experience it may be mentioned that neither hemorrhoids nor any draining lesion of the perineal region were noted. Serological examination of the blood and spinal fluids gave positive results to both the Hinton and Kahn tests, the latter had cell counts of 20 and 155 white and red cells respectively per cmm. The spinal fluid had 62 mgm. per cent total protein, of which globulin was 15 mgm. per cent and albumin 47 mgm. per cent, and the colloidal gold reaction was 5554321000. A diagnosis of meningoencephalitic syphilis with psychosis was made. From May 6th to June 24th he received 8 injections of Bismuth Sal. gr. ii. On re-examination in the subsequent November, after his psychosis had cleared, he gave a history of having had a draining "boil" to the left of the anus since 1942. Although not substantiated by the prior physical examination, this, if it ever existed, was probably a fistula.

The patient was admitted to the malariatherapy service in June, 1946, and on the 25th was inoculated with *P. falciparum* (Costa strain) by six positive mosquitoes of lot 778, and three positive mosquitoes from lot 780, a total of nine. Parasites were first observed on July 6th, and at 8 p.m. on the following day his temperature first exceeded 100°F. (101.1°F.), which initiated a period of remittent fever which continued until the 11th. On the 9th it exceeded 104°F. the whole morning, and transitorily this level at 8 p.m. on the 10th, but on neither day did it attain 105°F. During this period his parasite count slowly ascended to reach 2970 per cmm. on the 11th, a low density for infections with this parasite. He was given a single dose of quinine gr. iii at 4 a.m. on the 9th, when his

¹ The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Division of the Rockefeller Foundation, in cooperation with the Florida State Board of Health and the Florida State Hospital.

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temperature first exceeded 104°F., on which day he had a parasite count of 2250 per cmm. The temperature decline noted on the 11th initiated a brief period of tertian intermittency lasting until the 14th, but which on the afternoon of the 15th again became remittent, remaining, except at 4 p.m. on the 18th, continuously above 100°F. until the 20th, during which period it attained or exceeded 105°F. in the afternoons of the 16th and 17th, and 104°F. on the afternoon of the 19th. He experienced an intermission on the morning of the 20th, and thereafter a low remittent until the 26th, with afternoon rises which only once (23rd), exceeded 103°F. A low quotidian intermittent with afternoon elevations continued until the 29th. Thereafter until the 1st of August, it did not exceed 100°F. A second brief period of quotidian intermittent activity lasted until the 3rd, after which the fluctuations occurred within normal limits. Meanwhile the parasites rose to a low maximum of 8550 per cmm. on the 14th, on which day he had a temperature of 106°F. at 11 a.m., for which reason the small dose of quinine gr. iii was repeated. From this day the parasite density rapidly declined to 160, 200 and 10 on the 17th, 18th and 19th. We were mystified by the considerable temperature experienced in the face of a consistently low parasite density, and, fearing that perhaps the parasites might be, although unlikely, continuously remaining in the visceral circulation, quinine gr. x was given on the 18th. Giving consideration to the possibility of an intercurrent infection, a white count was made on the 18th, which revealed a leucocytosis of 13,300 per cmm., of which polymorphonuclears, lymphocytes and monocytes were respectively 78, 19 and 3 per cent. With this confirmation of the existence of an intercurrent infection, two further doses of quinine gr. x were given on the 19th to hasten the disappearance of the plasmodial parasitemia. The smear of the 20th was negative, and no malaria parasites were subsequently observed during the period (to Nov. 30th) in which the patient was on the service. A careful physical examination on the 19th revealed a crepitant cellulitis in the perineal region, extending to the left buttock and left side of the scrotum. On consultation with Dr. W. D. Rogers, Chief of the Medical Service, on that day, the immediate administration of penicillin and tetanus-gas gangrene antitoxin was decided upon.

On discovery of the cellulitis, the patient was taken to the operating room and the necrotic tissue was excised and a drainage tube inserted, and irrigation of the area with penicillin solution was initiated. Beginning on the same day, administration of 50,000 unit doses of penicillin intramuscularly every four hours was begun, which was continued until August 23rd when it was dropped to 25,000 units, and on September 6th again dropped to 15,000, and discontinued on the 27th of September. He received an approximate total of 19 million units.

On the 20th, an initial dose of 4 grams of sulfadiazine was given, initiating a course of 2 gram doses every 4 hours, which was continued until the 24th, for a total of 52 grams. Each dose was accompanied by a vii grain dose of sodium bicarbonate. On discontinuance, the patient had a plasma level of 6.9 mgm. per cent of sulfadiazine. A second course of sulfadiazine was begun on August 3rd,

and continued until the 15th, for a total of 118 grams, although on its completion, only a trace could be detected in the plasma.

A dose of 1,500 units of combined tetanus-gas gangrene antitoxin was given on the 19th, and two such doses were given daily to and including the 24th, when administration was temporarily interrupted, due to exhaustion of the stock. Administration was resumed on the 27th and continued until the 30th, two daily doses of gas gangrene antitoxin (10,000 units) being given daily.

On the 20th it was noted that there was some extension of the necrosis to the skin on the inferior surface of the penis, and the skin of the scrotum was incised and further drainage inserted. On the subsequent daily changes of dressings, the ulcerations were flushed with H_2O_2 and shreds of necrotic tissue were excised, and the cavity packed with gauze saturated with penicillin solution.

In the interval from the 20th of July to the 4th of August, the white counts varied from 7,700 to 12,900 per cmm.

On July 27th, the laboratory reported the identification of *Clostridium welchii* in culture made from serous exudate from the lesion.

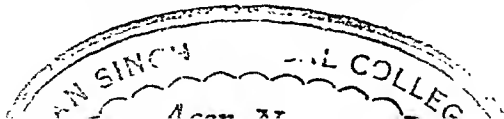
The patient's general condition remained critical until the 28th of July, although extension of the involved area had been checked, and abundant production of granulation tissue was beginning to fill the area of slough. His temperature subsided to normal on August 3rd, and until his discharge in November, did not exceed limits of normal variation. On the 8th of September skin from the thigh was grafted onto the granulated areas on the buttocks and scrotum. By the 13th of this month he could be permitted to go to the bath room, and dry dressings were substituted on the 18th. Early in November the patient was allowed to be completely ambulatory, and by the following March the perineal lesions were completely healed.

On November 18th the patient was re-examined, at which time the Kahn and Hinton reactions with the blood were negative. With the spinal fluid, the Hinton remained positive. Total protein in the spinal fluid was 68 mgm. per cent, of which albumin constituted 60 mgm. per cent, white and red cells were absent, and the colloidal gold test was completely negative. The patient was sufficiently well orientated in all planes to justify consideration for release on furlough, which was finally effected July 31, 1947.

In attempting appraisal of the role of the several elements of his experience during the period considered, those physicians who followed his progress subsequent to the 1st of November, formed the opinion that the massive penicillin therapy was probably the most important factor contributing to the improvement of his meningoencephalitic syphilis.

SUMMARY

A colored male patient with meningoencephalitic syphilis was inoculated with *Plasmodium falciparum* for malaritherapy. Although the inoculation was successful, the malaria infection was atypical, chiefly because the parasites did not attain densities proportional to the elevations of temperature experienced. Al-



though the plasmodial infection was subsiding spontaneously, it was therapeutically terminated on the 14th day of patency without interruption of the fever. It was thereupon discovered that the patient had a gas bacillus infection in the perineal region, which was finally overcome by the massive administration of pencillin, sulfadiazine and antitoxin. Recovery from the gas bacillus infection was complete, although healing of the area from which necrotic tissue had sloughed required several months, and was associated with marked improvement in the syphilitic infection. The plasmodicidal therapy administered could not have been sufficient to have aborted the malaria infection in the manner observed. It appears likely that the gas bacillus infection was active for some days prior to its discovery, and that the soluble exotoxin it produced may have adversely affected the plasmodia.

SOUTHWEST PACIFIC VIVAX MALARIA

CLINICAL FEATURES AND OBSERVATIONS CONCERNING DURATION OF CLINICAL ACTIVITY¹

EARL HILL, M.D.,² AND DONALD S. AMATUZIO, M.D.²

The purpose of this investigation is to review certain aspects of the problem of Pacific vivax malaria, symptomatology, phases of current treatment, relapse rate, and duration of clinical activity. Until recently the duration of clinical activity of vivax malaria was reported to be up to 2 years, with occasional cases persisting up to 3 years (1-8). Studies during and since World War II have shown that clinical activity may last 3 years or longer (9-12). Kekhcher (13), in a study of 5,500 cases of malaria in the Caucasian region of Russia, found that 1 to 4 per cent of vivax cases lasted 3 to 4 years.

A workable definition of the clinical duration of a case of malaria is the time elapsed from the initial attack (or the last attack in the endemic zone) until the final attack. A case cannot ordinarily be considered terminated unless no relapse has occurred in the preceding year. This qualification is in agreement with Russell and West's conclusion (3) that an individual cannot be told that he will not have another attack unless a year has passed without a relapse.

The cases in this study were treated with suppressive doses of atabrine for many months in the endemic zone and had therapeutic doses of atabrine and chloroquine repeatedly after return to the United States. There is good evidence that early therapeutic interference lengthens the duration of the infection (4, 7, 14). Boyd (15) states that intensive employment of drugs, in early stages before immunity develops, appears to be a factor in the recurrence of clinical activity so often noted. Boyd (16) and Sapero (17) feel that the reason early treatment increases the likelihood of further episodes is that all fixed tissue cell stages have not been discharged into the circulation and are therefore retarded in development.

It is recognized that Pacific vivax malaria represents a complex situation in that each patient frequently harbors multiple strains which are not synchronized. This consideration has not been adequately stressed in the literature on Pacific vivax infection.

MATERIAL

This study extended over the period July 1, 1945 to April 1, 1948. During this period 328 cases of Southwest Pacific vivax malaria were studied at this hospital. All subjects had received suppressive doses of atabrine while in the Pa-

¹ Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the authors.

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cific. Suppressive medication was usually discontinued on return to the United States. All cases were diagnosed by blood smear at this hospital. The patients were young, white World War II veterans, in otherwise good physical condition. They had served in the South, Southwest and Central Pacific and in the Philippine Islands. Since discharge from Service these veterans have all resided in a non-malarial area (Minnesota, North Dakota, South Dakota, and Wisconsin). There was practically no possibility of reinfection with vivax malaria after leaving the Pacific.

TABLE I
Less well recognized prodromal symptoms (239 cases)

SYMPTOMS	NUMBER	PER CENT
One or more gastro-intestinal symptoms.....	45	18.8
Vomiting.....	33	13.8
Nausea.....	29	12.1
Abdominal cramps.....	14	5.8
Diarrhea.....	12	5.0
Anorexia.....	11	4.6
Epigastric pain.....	5	2.1
One or more central nervous system symptoms.....	26	11.0
Headache.....	21	8.7
Dizziness.....	12	5.0
Retro-bulbar pain.....	7	2.9
Fatigue.....	6	2.5
Confusion, delirium.....	2	0.8
One or more respiratory symptoms.....	10	4.0
Cough.....	9	3.8
Chest Pain.....	5	2.1
One or more musculo-skeletal symptoms.....	43	18.0
Low back pain.....	27	11.3
Weakness.....	21	8.7
Migratory joint pain.....	14	7.4
Bone pain.....	9	3.8

SYMPTOMATOLOGY

The usual picture of chills, fever, and malaise was regularly encountered. Attention is directed to less well recognized prodromal symptoms occurring before the manifest chills and fever. These symptoms have been noted by previous investigators [Most (18), Gordon and Lippincott (19), Kaplan (20)]. The incidence of these less well recognized prodromal symptoms in this study is indicated in Table I. The prodromal symptoms occurred 2 to 14 days before the disease was recognized as malaria. One hundred twelve out of 239 cases (46 per cent) had one or more of these symptoms. These symptoms made differential diagnosis difficult in the prodromal period. Dysentery, hepatitis, encephalitis, rheumatic fever, and other infectious diseases were frequently considered possibilities at this stage.

PHYSICAL FINDINGS

Except for fever, the essential physical findings were confined to the spleen and liver. Previous investigators have found an incidence of splenomegaly ranging from 9 to 80 per cent [Boyd (21) 80.4 per cent; Gordon (19) 23 per cent; Barber and Rice (22) 33.2 per cent; Hackett (23) 52.4 per cent; Barber (24) 68.5 per cent]. Hepatomegaly has been commented upon previously by Engstrom *et al.* (25) and Kern and Norris (26). In this study 37.6 per cent of the subjects had palpable spleens of which 27.7 per cent were tender (Table II). The greatest splenic enlargement found in this study was 4 cm. below the costal border. In most of the subjects the splenomegaly was only 1 or 2 cm. below the costal border. The marked splenomegaly found in poorly nourished individuals in the tropics subject to multiple re-infections with malaria was absent in these patients.

TABLE II
Splenomegaly and hepatomegaly (239 cases)

CM. BELOW COSTAL BORDER	NO. PALPABLE	PER CENT PALPABLE	PER CENT TENDER
Splenomegaly			
1	65	27.1	
2	16	6.7	
3 or more	9	3.7	
Total.....	90	37.6	27.7
Hepatomegaly			
1	25	10.5	
2	7	2.9	
3 or more	4	1.7	
Total.....	36	15.1	50

Thirty-six patients (15.1 per cent) had palpable livers, of which 50 per cent were tender. Enlargement of both the liver and spleen was found in 29 cases (12.1 per cent). Enlargement of the liver without enlargement of the spleen was found in 7 cases (3.0 per cent) of whom 5 had liver tenderness. One case had only right upper quadrant tenderness without organ enlargement, and 3 cases had left upper quadrant tenderness without organ enlargement. Three cases had a moderate generalized adenopathy. Four cases (1.7 per cent) presented jaundice, a manifestation of malarial hepatitis. Two and one-tenth per cent of the cases in the present study had herpes labialis.

LABORATORY FINDINGS

Each relapse was associated with the finding of *Plasmodium vivax* in the blood smear. The thick smear method was used routinely, supplemented by thin smear when necessary to identify the species. Twenty-three (9.6 per cent) of

239 subjects had a hemoglobin level of 12.0 grams or less. Three patients had a hemoglobin of 7.5 grams or less. The hemoglobin level was not related to the number of attacks. The average hemoglobin value for 59 patients observed in their first attack was 14.0 grams. The average hemoglobin for 17 subjects observed in their tenth or higher attack was 14.5 grams. It is apparent that multiple relapses of malaria in well nourished individuals do not result in anemia.

Leukopenia and monocytosis have been described in malaria previously (27). A leukocyte count of 5,000 or less was encountered in 24.3 per cent of the subjects (Table III). A lymphocytosis of 40 per cent or over, was noted in 31 per cent of the cases. Monocytosis was noted in 25 subjects (12.2 per cent), the range of monocytes being from 5 to 20 per cent. Three subjects (1.4 per cent) had an eosinophilia of 5 per cent or over. None of these patients had accompanying intestinal parasitism. One patient had an eosinophilia of 22 per cent which declined to normal under therapy. Erythrocyte sedimentation rates were determined in 57 cases, 66 per cent of which were elevated (taking 20 mm. in one hour as the upper limit of normal).

TABLE III
Hemoglobin and leukocyte counts (239 cases)

HEMOGLOBIN (NORMAL: 15.4 GM.)			LEUKOCYTES		
Grams	No. of cases	Per cent	W.B.C.	No. of cases	Per cent
5.5- 9.0	5	2.1	1000-3000	4	1.7
9.1-12.0	18	7.5	3001-5000	54	22.6
12.1 and above	216	90.4	5001 and above	181	75.7
Total.....	239	100.0		239	100.0

THERAPY

The two drugs used in this study were atabrine and chloroquine. The dosage of atabrine used was 0.2 grams every 6 hours for 5 doses, then 0.1 gram three times daily for 6 days, a total of 2.8 grams. The dosage of chloroquine diphosphate was 1.0 gram initially, 0.5 gram in 6 hours, then 0.5 gram on the second and third days. A total of 169 cases were treated with atabrine and 62 with chloroquine. No toxic manifestations were noted with either drug. The cases were followed for an average of 2 years.

The average number of attacks per person prior to therapy at this hospital was 2.8 attacks for the atabrine treated cases and 4.4 attacks for the chloroquine treated cases. Of the atabrine treated patients, 110 (65 per cent) experienced relapses, having a total of 213 relapses, or an average of 1.9 attacks. Thirty-five (56 per cent) of the chloroquine treated cases experienced relapses, having a total of 59 relapses, or an average of 1.7 attacks.

The atabrine treated group had 66 per cent of its relapses in the first 180 days. The chloroquine treated group had 94 per cent of its relapses in that same period, as indicated in Table IV. Not until 360 days did the atabrine group have an

equivalent percentage (95 per cent) of relapses. It should be noted that a substantial percentage of relapses occurred beyond 360 days in the atabrine group. Figure 1 is a graphic representation of the occurrence of relapses following treatment by the two drugs.

To make a truer comparison of atabrine and chloroquine, a group of atabrine treated patients was selected in which the average number of attacks prior to therapy at this hospital (4.2) was approximately the same as for the chloroquine treated group (4.4). In this atabrine treated group the relapse rate was 60 per cent. The chloroquine treated group had a relapse rate of 56 per cent. The difference between these two percentages is not statistically significant. In this atabrine treated group the relapses were even further delayed than in the whole atabrine group.

TABLE IV
Relapses following treatment

AVERAGE ATTACK TREATED	ATABRINE 2.8			CHLOROQUINE 4.4		
	No. of cases treated: 169	No. relapsed: 110	Per cent relapses: 65	No. of cases treated: 62	No. relapsed: 35	Per cent relapsed: 56
	Occurrence of relapse		Cumulative per cent	Occurrence of relapse		Cumulative, Per cent
	No.	Per cent		No.	Per cent	
<i>days</i>						
0- 89	64	30	30	40	67	67
90-179	75	36	66	15	27	94
180-269	45	21	87	2	3	97
270-359	18	8	95	2	3	100
360-449	5	2	97			
450-539	4	2	99			
540-629	2	1	100			
Total.....	213	100	100	59	100	100

The average number of hours of fever following institution of chloroquine was 16.9. With atabrine the average was 22.4 hours. The distribution curve of the number of hours of fever had a modal value of 12 hours for chloroquine and 16 hours with atabrine.

DURATION

An attempt was made to study the duration of Southwest Pacific vivax malaria, regardless of therapy. Of special interest were the relation of the relapse rate to the number of the attack and the duration of clinical activity. It was particularly feasible to study the duration of clinical activity in this group because the veterans resided in non-endemic areas since return from the Southwest Pacific. Malaria is essentially non-existent in the North Central states (28, 29). No attempt was made to distinguish between recrudescence and true relapse, as defined by Boyd and Kitchen (7).

a. Relapse rate versus the number of the attack

Dieuaide (30) found that 70 to 80 per cent of cases of Southwest Pacific vivax malaria had a first relapse. He predicted that only 0.1 per cent or less of the cases would have 10 to 15 relapses. He also noted a progressive decrease in the per cent of each relapse group that had further recurrences. Russell, West and Manwell (3) state that less than 5 per cent have 5 relapses and only 0.5 per cent have 8 or more. In the present study, the relapse rate in progressive attacks of 222 cases was analyzed. A first relapse occurred in 75 per cent of the cases, a

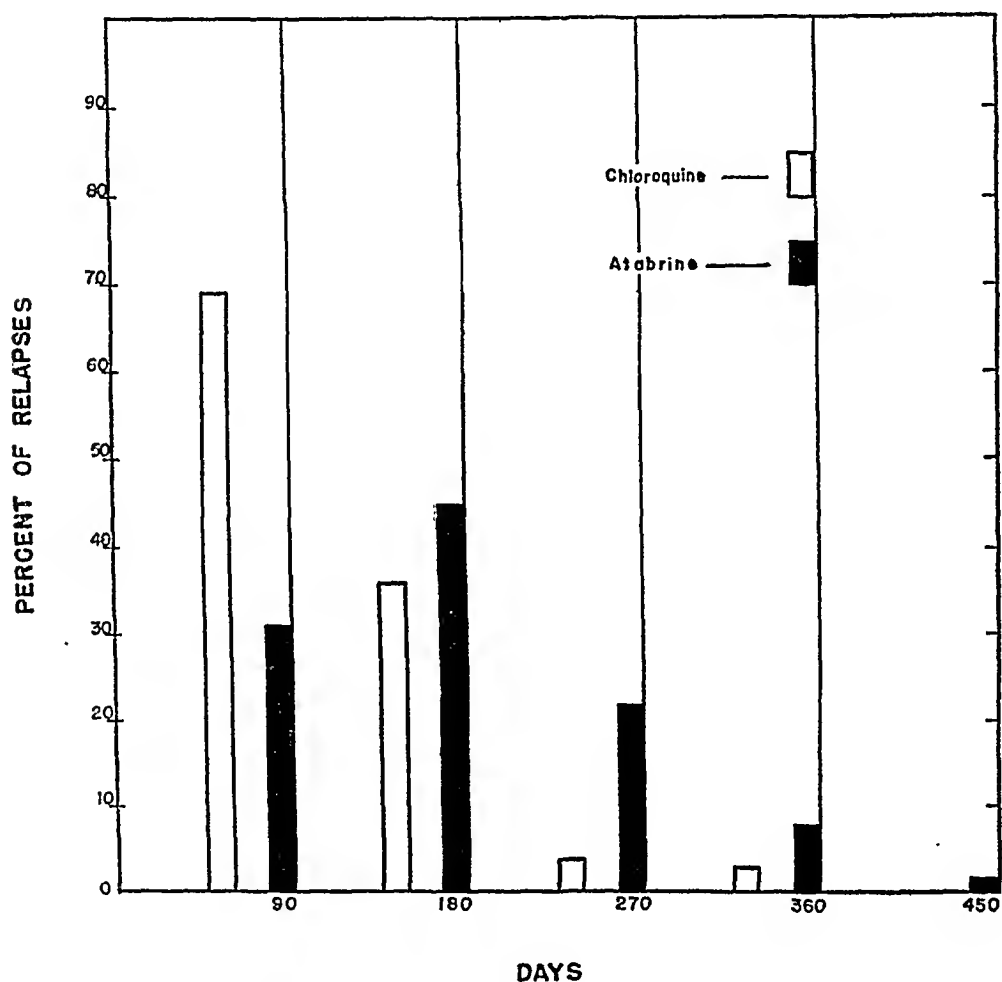


FIG. 1. OCCURRENCE OF RELAPSES FOLLOWING TREATMENT

second relapse in 65 per cent, and a third relapse in 47 per cent. A fifth relapse occurred in 26 per cent, a tenth in 9 per cent, and a fifteenth in 0.4 per cent (fig. 2). The per cent of each relapse group that had further recurrences varied from 62 to 88 per cent, there being no definite sequence similar to that noted by Dieuaide. This is in agreement with Boyd's experience (7) with both spontaneously and therapeutically terminated vivax malaria. It is evident that Dieuaide's prediction that 10 to 15 relapses would occur in 0.1 per cent or less of the cases is too conservative.

b. Duration of clinical activity

In 155 cases it was possible to study the total course to determine the duration of activity. The initial point of the disease was taken to be the first attack with a positive smear. [The cases with a large number of attacks usually had their earlier attacks overseas so that the data concerning their attacks while overseas had to be accepted from the patients' histories. Of those relapses included but not treated at this hospital, only those were included which the patient specifically stated were associated with a positive blood smear.] Each case was laboratory proven at this hospital. A positive smear was also obtained at this hospital for the final attack in each case. These cases were followed for an aver-

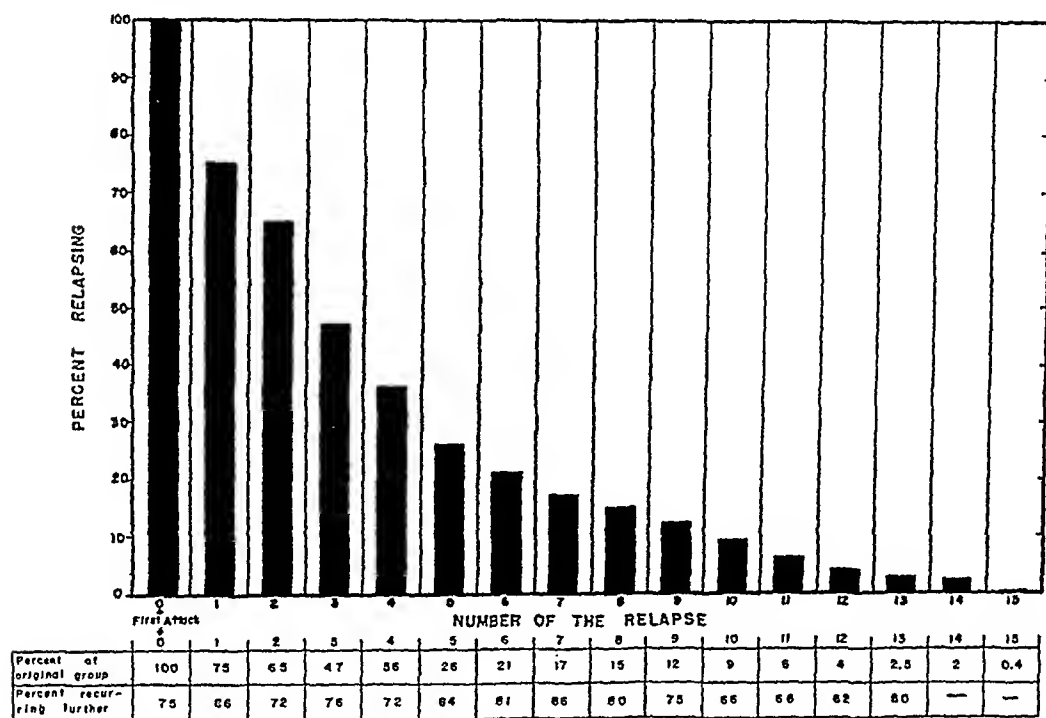


FIG. 2. CHART ILLUSTRATING PER CENT OF ORIGINAL GROUP HAVING PROGRESSIVE RELAPSES, AND PER CENT OF EACH RELAPSE GROUP RECURRING FURTHER (222 CASES)

age of two years. Of this group recurrences were limited to one year in 38 (24.5 per cent), to two years in 82 (52.9 per cent), to three years in 28 (18.3 per cent), and to four years in 7 (4.3 per cent). Thus, the disease exceeded one year in 117 cases (75.3 per cent) and two years in 35 cases (22.6 per cent) (Table V).

The frequency distribution of the duration of these cases of malaria is given in figure 3. This distribution has a mean of 16.5 months and a standard deviation of 11.5 months. The likelihood of having a case last beyond 36 months is small but definite (4.3 per cent in this study). A majority of the cases persisted into the second or third year, the frequency being highest in the range of 12 to 30 months duration. Each case was followed-up by questionnaire for 12 months after the last relapse to determine if it could validly be considered termi-

nated. Of the 7 cases presented in the fourth year of the disease, 3 had relapses within the last six months, and thus cannot actually be said to be terminated.

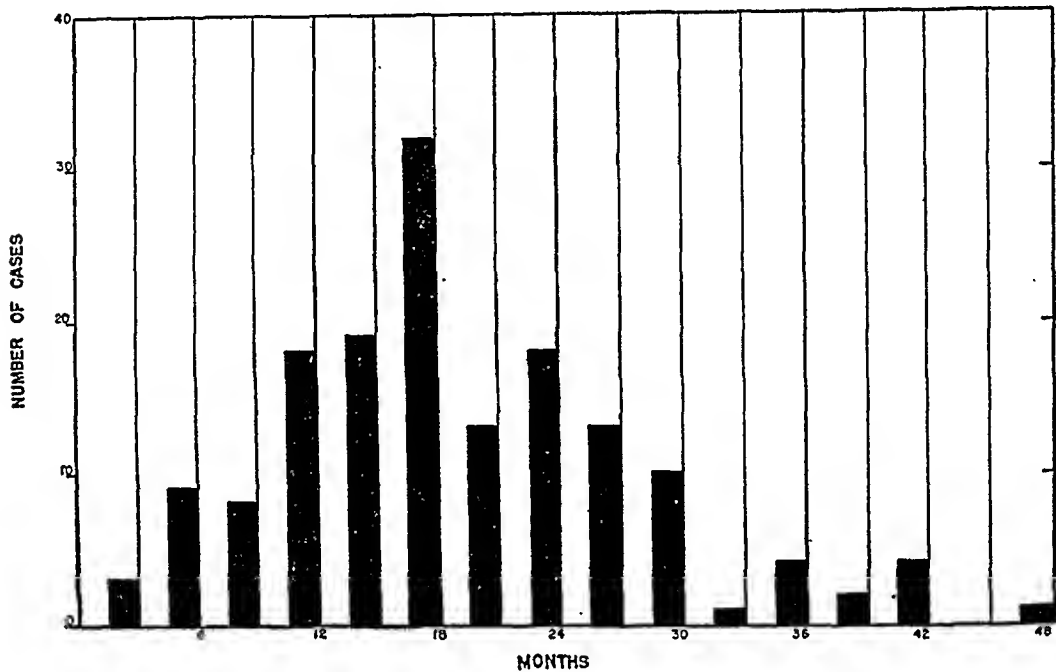


FIG. 3. FREQUENCY DISTRIBUTION OF THE DURATION OF MALARIA (155 CASES)

TABLE V
Duration of disease

YEAR	NUMBER OF CASES	PER CENT
1st	38	24.5
2nd	82	52.9
3rd	28	18.3
4th	7	4.3
Total.....	155	100.0

DISCUSSION

The prodromal symptoms listed made the diagnosis difficult in instances of the primary attack until the typical pattern of chills and fever was manifest. Marked splenomegaly was absent and marked anemia was rare.

Dealing with multiple strain *Pacific vivax* malaria similar to this study, previous investigators found a relapse rate following atabrine of 67 to 84 per cent (Table VI). Gordon *et al.* reported a relapse rate following atabrine of 74 per cent in April 1946 (31), and 67 per cent one year later (32). In the first report on the clinical use of chloroquine, Most *et al.* (33) found a relapse percentage of 75 per cent. From his study it was concluded that chloroquine relapses occur

later than relapses after atabrine. The superiority of chloroquine in this regard was not borne out by Gordon *et al.* (32) or by the present study.

In the present series the total relapse rate over a 2-year follow-up period was 65 per cent for atabrine and 56 per cent for chloroquine. In groups paired with respect to average attack treated the difference in relapse rates was even less (60 per cent for atabrine and 56 per cent for chloroquine) and is statistically insignificant. Chloroquine relapses occurred much earlier. In the first 90 days (Table IV) there were over twice as many relapses with chloroquine as with atabrine. At the end of 180 days, the chloroquine group still had 1.5 times the percentage of relapses, as did the atabrine group. Not until one year of follow-up had the bulk of relapses following atabrine occurred. Neither chloroquine nor atabrine cure malaria but atabrine would appear to be superior to chloroquine in that relapses occur later after the use of atabrine.

TABLE VI

Relapse rates in Pacific malaria reported by various authors

AUTHOR	DATE OF STUDY	ATABRINE		CHLOROQUINE		AVERAGE FOLLOW-UP PERIOD
		Average attack	Relapse percentage	Average attack	Relapse percentage	
						<i>mos.</i>
Gordon <i>et al.</i> (31).....	Apr. 1946	—	74	—	—	7
Most <i>et al.</i> (33).....	July 1946	—	82	—	75	3
Most <i>et al.</i> (35).....	Oct. 1946	4.8	84	—	—	3
Gordon <i>et al.</i> (32).....	Apr. 1947	*	67	*	75	3
Warthin <i>et al.</i> (10).....	Apr. 1948	—	84†	—	84†	1-12
			42‡		42‡	
Present study.....	—	2.8	65	4.4	56	24
Present study§.....	—	4.2	60	—	—	24

* "Secondary attacks."

† "Clinical relapses."

‡ "Laboratory relapses."

§ Atabrine group selected on basis of average attack treated.

As far as can be determined, long term observation of Pacific vivax malaria for relapses and duration of the infection has not been feasible in the past. Studies in the service were limited in their follow-up to approximately 120 days [Most (33); Gordon *et al.* (32)]. Several authors [Dieuaide (30), Bianco *et al.* (34), Warthin (10)] had opportunity for up to one year observation, but none studied cases in their total clinical duration. A common statement in the literature was that only 1 per cent of the cases would have relapses beyond 2 years.

Of 155 cases studied for duration, none was treated with drugs which might reasonably be expected to cure the infection, namely a plasmochin-quinine combination (35) or a pentaquine-quinine combination (36, 37). The majority of the cases in this study persisted into the second and third year of the disease. Roughly one-fifth of the cases had relapses in the third year; 4 per cent were still

having relapses in the fourth year; 2 per cent had a good possibility of still being active in the fifth year. Thus it is only in the fifth year after initial infection that relapses become rare. The earlier impression that few cases would last beyond two years is not borne out by these data.

The clinical features of this group of cases should be interpreted as the characteristics of multiple strain infections and possibly superposed infections of homologous strains. Boyd and Kitchen demonstrated 2 strains of the Southwest Pacific vivax parasite in a returned veteran (38). Different strains vary characteristically in the frequency of secondary episodes (39). Boyd, Kupper and Mathews (40) showed that homologous immunity to vivax malaria is more slowly acquired when two strains are simultaneously inoculated. The complexity of the presence of multiple strains is such that the features of this study are only significant in a clinical light, and cannot be applied to the duration or study of immunity of a single strain infection.

SUMMARY

The symptomatology, duration, relapse rate, and response to treatment of Southwest Pacific vivax malaria was evaluated in 328 veterans who were studied over a 33-month period. The infections were diagnosed by blood smear.

Prodromal symptoms other than chills, fever and malaise were elicited in 46 per cent of the cases.

Splenomegaly was noted in 37.6 per cent, and hepatomegaly in 15.1 per cent of the cases.

In groups paired on the basis of average attack treated, the relapse rate following chloroquine was 56 per cent and atabrine 60 per cent. The relapses following chloroquine occurred much earlier. Chloroquine has no advantages over atabrine.

In the present series the relapse rate decreased with further attacks but the per cent of each relapse group recurring further was approximately the same from one attack to the next.

Of a group of cases studied in the entirety of their clinical duration, 24.5 per cent were terminated in the first year, 52.9 per cent in the second year, and 18.3 per cent in the third year. Attacks were still occurring in 4.3 per cent in the fourth year of the disease. The frequency distribution of the duration of this group of cases is presented.

Relapses of Pacific vivax malaria will be a rarity in the fifth year after infection. The earlier impression that few cases would last beyond two years is not borne out by this study.

Patients with Pacific vivax malaria often harbor multiple strains. The duration of clinical activity of such a group of cases should not be interpreted as the behavior of a single strain infection.

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POTENTIAL VECTORS OF JAPANESE ENCEPHALITIS IN THE CAROLINE ISLANDS¹

HERBERT S. HURLBUT² AND JOHN I. THOMAS³

INTRODUCTION

The discovery of Japanese encephalitis on the island of Guam by Hammon (1) makes it seem probable that this disease will be found on other tropical islands of the Pacific. Recently an opportunity was afforded to test the ability of two species of mosquitoes from Ponape, Eastern Caroline Islands, to transmit the virus. While the disease is not known to occur on Ponape, it seemed worth while to investigate as potential vectors the indigenous species of mosquitoes which most commonly bite man.

MATERIALS AND METHODS

The brains of albino mice, which died of the disease four to five days after intracerebral inoculation, were emulsified in water containing ten per cent cane sugar and this material was fed to the females of laboratory reared mosquitoes. *Culex quinquefasciatus* Say, and *Culex annulirostris* Skuse were the species tested. The virus used was the Okinawan strain isolated by Hodes, Thomas and Peck in 1945 (2). After an extrinsic incubation period of six to eight days at 30°C. the mosquitoes were permitted to bite suckling mice four to seven days old. The brain of each mouse which became ill was removed aseptically and a ten per cent suspension was inoculated intracerebrally into 21-day old mice. If the brain proved to be bacterially sterile and the passage mice developed typical symptoms and died in four to six days, mosquito transmission of the virus was considered to have been effected. In the case of *C. annulirostris*, the identity of the virus in the mosquito-infected mouse was proved by neutralization test. Further proof of the presence of virus in the mosquito was obtained by introducing a saline suspension of the ground up mosquitoes into mice intracerebrally.

RESULTS

The results are summarized in table 1. Only a small number of *C. annulirostris* were available for experimentation and these did not feed well either on the infective material or the test mice. However, out of ten mice exposed to the mosquitoes one became ill with typical symptoms including convulsions on the fifth day. The brain was bacterially sterile and harbored the virus of Japanese encephalitis in high titer as shown by neutralization test. After 15 days the three surviving specimens of *C. annulirostris* were emulsified in saline in a di-

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lution of approximately 1 to 50, the emulsion was centrifuged at 2000 rpm. for ten minutes and mice were inoculated intracerebrally. Four out of five of these mice became ill on the sixth day. One brain was passaged and cultured for bacteria. It contained a small number of nonpathogenic forms. The second passage mice died in four to six days. One brain was cultured and found to be

TABLE 1
The transmission of Japanese encephalitis by mosquitoes

LOT	INFECTIVE MATERIAL	MOSQUITO	EXTRINSIC INCUBATION DAYS, 30°C.	TRANSMISSION, NO. TIMES EFFECTED		CONFIRMATION
				By bite	By inoculation of emulsified mosquitoes	
1	Mouse brain 1-10 dilution	<i>Culex annulirostris</i>	7	1		By neutralization test. Index 30,000
1	" "	<i>Culex annulirostris</i>	16		1	By neutralization test. Index 4,000
2	" "	<i>Culex quinquefasciatus</i>	6	1		By passage
3	" "	<i>Culex quinquefasciatus</i>	8	1		By passage

TABLE 2
Mosquitoes caught while attempting to bite man, Ponape, Eastern Caroline Islands

DATE	HOUR	PLACE	SPECIES	
			<i>C. quinquefasciatus</i>	<i>C. annulirostris</i>
12 Feb	1930-2000 2130-2200	Campsite	14	5
13 Feb	1900-1930 2130-2200	Campsite	20	4
15 Feb	1900-1930	Campsite	13	4
18 Feb	1900-2000	Mechikku	5	15
28 Feb	1930-2000	Campsite	10	0

bacterially sterile. By neutralization test, this brain also was shown to harbor the virus of Japanese encephalitis in high titer.

The laboratory transmission of Japanese encephalitis to mice by *C. quinquefasciatus* has been reported by Reeves and Hammon (3). The present tests confirm their results. In the first test a five-day old mouse was bitten by six mosquitoes after an extrinsic period of six days at 30°C. The mouse developed convulsions on the fifth day. The brain proved to be bacterially sterile and all of the five passage mice used died in four to six days after intracerebral inocu-

lation. In the second test a four day old mouse was bitten by 14 mosquitoes after an extrinsic period of eight days at 30°C. The mouse developed typical symptoms on the fifth day. The brain was bacterially sterile and all of the five mice used became prostrate on the fourth day after intracerebral inoculation.

COMMENTS

C. quinquefasciatus has a world-wide distribution in tropical and subtropical areas. It is a common mosquito about human dwellings on most of the islands of the Central Pacific (4, 5). It is nocturnal in its habits, feeding readily on man from soon after sunset until dawn (table 2).

C. annulirostris occurs in northern Australia, Samoa, Tonga, Ellice Island, Fiji, New Hebrides, Solomon Islands, New Zealand, New Guinea, Caroline Islands, Moluccas, Lesser Sandas, Sumatra, Celebes, Borneo, and the Philippines, according to Bohart and Ingram (4). According to these workers it is a serious nocturnal pest on the islands of the Truk group. On Ponape this mosquito was observed by the authors to bite man readily (table 2) and it feeds commonly also on the domestic pig. A trap modified from that of Magoon (6) baited with a live pig attracted this species in large numbers.

SUMMARY AND CONCLUSIONS

1. Tests were made to determine whether the mosquitoes, *Culex quinquefasciatus* Say and *Culex annulirostris* Skuse are able to transmit the virus of Japanese encephalitis to mice.

2. The mosquitoes were infected by being fed an emulsion of infected mouse brain. Young mice bitten by these mosquitoes developed symptoms typical of encephalitis. The brains of these mice were shown to contain the virus of Japanese encephalitis in high titer by passage and by neutralization tests.

3. These two species of mosquitoes bite man commonly and it is concluded that they should be considered potential vectors of Japanese encephalitis which is now known to occur on Guam and is possibly present on other tropical islands of the Pacific.

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REPORT ON THE PRESENCE OF JAPANESE B ENCEPHALITIS NEUTRALIZING ANTIBODY AMONG FILIPINOS AND CERTAIN PHILIPPINE ANIMALS¹

E. S. SALAFRANCA, D.V.M. AND L. ESPIRITU, M.D.²

Due to the presence of Japanese B encephalitis in the Japanese island groups, and since heavy interchange of military personnel had occurred between those islands and the Philippine Islands, it was deemed advisable to make a survey of this disease in the Philippines. The survey was started by the 19th Medical General Laboratory in Manila, now 3rd Medical General Laboratory. In preparing for the survey, Captain Nathan Hale, MC, and one of the authors (E. S. S.) were sent to the Virus and Rickettsial Disease Laboratory in Tokyo for training in technical procedures by the Commission.

MATERIALS AND METHODS

As originally planned serum neutralization tests and complement fixation tests would be used, however, due to shortage in antigen only a portion of the complement fixation tests could be completed.

The Nakayama strain of virus was obtained from the Department of Virus and Rickettsial Disease AMDRGS, Washington, D. C. and was used throughout all tests. Laboratory bred and raised 21-30 day white mice were used. For diluting the virus inactivated rabbit serum, free from neutralizing antibodies was used in proportion of 1 to 10 with isotonic solution of sodium chloride. The technique for the neutralization tests was that employed at the Virus and Rickettsial Disease Commission Laboratory in Tokyo, except as noted. A 10 per cent suspension of three or more fresh mouse-passage-virus brains was prepared with 50 per cent rabbit serum in physiological saline and thoroughly emulsified. This was centrifuged at 2000 rpm for 10 minutes. The supernatant fluid was serially diluted with 10 per cent rabbit serum in isotonic sodium chloride solution and preliminary titrations made for the LD₅₀ potency of the virus. In the actual tests and simultaneous virus titrations, the virus dilutions were adjusted to the LD₅₀ obtained in the preliminary titrations to meet requirements. The dilutions for the control series were adjusted to include the lowest dilution which was expected to cause 100 per cent mortality in the mice inoculated and the highest dilution in which 100 per cent survival was expected. Those for the test proper were adjusted to enable detection of a neutralization index of 50 or more.

¹ This work was started at the 19th Medical General Laboratory under the command of Colonel D. M. Kuhns, MC. The complement fixation tests were performed by Dr. F. San Agustin of the Serology Department. Edited and correlated by Colonel H. R. Livesay, MC, and Capt. C. A. McWhorter, MC, of the 3rd Medical General Laboratory. The authors acknowledge with gratitude the invaluable assistance of Dr. V. Basaca of the Serology Department, 3rd Med. Gen. Lab. in performing the neutralization tests on the specimens presented in Table V.

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The serum-virus mixtures were incubated at 37°C. in a water bath for two hours and stored at 5°C. until inoculated. The mice were anesthetized with ether and 0.03 cc. of the mixture inoculated intracerebrally with a $\frac{1}{4}$ inch, 25-gauge needle on a 1-cc. tuberculin syringe. The highest dilutions were inoculated first, down to the lowest. Five mice were used for each dilution. Rabbit serum free from neutralizing antibody was used for the negative control.

A variation in the procedure which was employed in some of the tests was holding the serum-virus mixtures for from 18–20 hours at refrigerator temper-

TABLE I
Neutralization tests on Filipino civilians

SPECIMEN	AGE	GEOGRAPHIC TRAVELS	NEUTRALIZATION INDEX
	<i>years</i>		
F-1	27	Luzon	317
F-2	27	Luzon	3
F-4	24	Luzon	10
F-5	24	Luzon	31
F-6	29	Luzon	10
F-7	27	Luzon and Mindanao	395
F-8	25	Luzon	31
F-9	26	U. S. A., Luzon and Mindanao	10
F-10	31	Luzon and Visayas	317
F-11	27	Luzon and Visayas	100
F-12	48	Luzon	79
F-13	27	Luzon	10
F-15	18	Luzon	10
F-16	19	Luzon	317
F-17	25	Luzon and Europe	10
F-18	21	Luzon	10
F-19		No data obtainable	10
F-21	21	Luzon, Mindanao and Visayas	10
F-23	19	Luzon	10
F-24	20	Luzon	10
F-25	19	Luzon	126

ature (approximately 5°C.) instead of at 37°C. for two hours in the water bath. In these instances, previously tested positive and negative controls were included. The titer of the virus, in these controls, was invariably the same with both methods.

Deaths during the first 3 days were attributed to non-specific causes. The mice were observed for a period of 14 days. The LD₅₀ was determined by the method of Reed and Muench (1). The neutralization index was computed as the ratio of the LD₅₀ for the control over that for the test mice.

The technique of Casals and Palacios (2) for the complement fixation tests was employed in testing the sera of the Japanese POW's and a few suspicious cases of Japanese B encephalitis infection. The antigen was prepared in the Virus Laboratory in Tokyo.

TABLE II
Neutralization tests on Japanese prisoners of war

SPECIMEN	AGE	GEOGRAPHIC TRAVELS	NEUTRALIZA- TION INDEX	COMPLEMENT FIXATION TITER
	<i>years</i>			
J-1	24	Luzon (2 yrs.) Honshu (21 yrs.) Korea (1 yr.)	100,000	2+ (1:8)
J-2	34	Honshu (30 yrs.) Hokaido (1 yr.) Shanghai and Saipan (1 yr.) Luzon (2 yrs.)	100,000	Negative
J-3	20	Honshu (18 yrs.) Luzon (2 yrs.)	100,000	2+ (1:8)
J-4	27	Honshu (21 yrs.) China (4 yrs.) Luzon (2 yrs.)	2,000	2+ (1:2)
J-5	28	Honshu (26 yrs.) Luzon (2 yrs.)	100,000	Negative
J-6	24	Honshu (22 yrs.) Luzon (2 yrs.)	6,400	2+ (1:8)
J-7	36	Honshu (33 yrs.) Luzon (3 yrs.)	100,000	Negative
J-8	23	Honshu (20 yrs.) China (1 yr.) Luzon (2 yrs.)	100,000	3+ (1:16)
J-9	24	Kyushu (22 yrs.) Luzon (2 yrs.)	32,000	Negative
J-10	20	Kyushu (16 yrs.) Luzon (4 yrs.)	260	2+ (1:16)
J-11	33	Honshu (31 yrs.) Luzon (2 yrs.)	10	Negative
J-12	30	Honshu (28 yrs.) Luzon (2 yrs.)	100,000	2+ (1:16)
J-13	29	Honshu (21 yrs.) China (2 yrs.) Manchouko (1 yr.) Korea (1 yr.) Formosa (1 yr.) Luzon (2 yrs.)	10	Negative
J-14	27	Honshu (21 yrs.) Manchouko (1 yr.) China (1 yr.) Formosa (1 yr.) Luzon (2 yrs.)	10	Negative
J-15	27	Honshu (21 yrs.) Manchouko (2 yrs.) China (1 yr.) Formosa (1 yr.) Luzon (2 yrs.)	16,000	2+ (1:2)
J-16	30	Honshu (25 yrs.) Manchouko (3 yrs.) Luzon (2 yrs.)	160	Negative
J-17	22	Kyushu (18 yrs.) Shikoku (1 yr.) China (1 yr.) Luzon (2 yrs.)	80	Negative

RESULTS

Specimens of serum were collected and tested from 21 normal adult Filipinos (Table I); 17 Japanese prisoners of war attached to this unit (Table II); 13 ill patients suspected of being cases of encephalitis (Table III); 29 Philippine cattle (Table IV); 23 horses (Table VII); 11 carabaos (water buffaloes) (Table V) and 8 Philippine goats (Table VI).

The interpretation of the results of the neutralization tests was based on the criteria of Paul (3) and Sabin (4). The results tabulated below were based on

Paul's criteria, in that, only those sera with neutralization index above 50 were considered positive.

Comparative results

SPECIMEN	INDEX	
	A.M.D.R. and G.S.	3rd M.G.L.
Geslani, C., 9 July 1946.....	200	10,000
Sison, L., 10 July 1946.....	200	2,000
P.O.W. #3.....	630	100,000
P.O.W. #5.....	80	100,000
P.O.W. #12.....	3,200	100,000
Cereza, T., 23 October 1946.....	1,200	5,630
Cattle #2.....	10	50
Cattle #21.....	10	63
Jacob, J., 11 February 1947.....	1,600	252
Jacob, J., 4 March 1947.....	1,000	3,170
Carabao #6.....	1,000	3,170
Carabao #15.....	1,000	3,170
Carabao #31.....	1,600	3,170

In order to check on our results samples of the sera tested, of which we had sufficient amounts, were sent to the Department of Virus and Rickettsial Diseases, Army Medical Department Research Graduate School in Washington. The results reported (5, 6, 7) were, as a general rule, lower than those obtained by us, with one exception. This was expected because of the time element and the adverse storage condition before we had an opportunity of sending them to Washington, D. C.

Consolidated results

	TOTAL	NEGATIVE	POSITIVE
Normal Filipinos (Table I).....	21	14	7
Japanese P.O.W. (Table II).....	17	3	14
Suspected cases of Japanese B Encephalitis (Table III).....	13	6	7
Philippine cattle (Table IV).....	29	16	13
Philippine carabaos (Table V).....	11	1	10
Philippine goats (Table VI).....	8	6	2
Philippine horses (Table VII).....	23	2	21

DISCUSSION

The normal adult Filipino human group gave 33.3 per cent positive as compared with 82 per cent positive in the Japanese group. Eleven of 14 or 78 per cent of the Japanese from Honshu gave positive indices which could be interpreted as an indication of the extent of the infection in that Island, granting

TABLE III
Suspected number cases of Japanese B Encephalitis

AGE	SEX	RACE	CLINICAL MANIFESTATIONS	DATE OF SPECIMEN	NEUTRALIZATION INDEX	COMPLEMENT FIXATION TITER
Yrs.						
21	M	F†	Onset—May 30 '46. Fever, headache, muscular pain, diplopia, delirium, stiffness of neck, positive Kernig—bilateral; positive Babinski—bilateral.	1st—12 Jun. '46 2nd—27 Jun. '46	39 317 2000	Negative 3+ (1:16) N. T.*
34	F	F	Onset—May 24 '46. Fever, choreic movements of fingers and toes, grimaces, stiffness of body, incontinence, severe headache; mentally confused	1st—10 Jun. '46 2nd—26 Jun. '46 3rd—9 Jul. '46	5 2040 10000	N. T. N. T. 3+ (1:16)
12	F	F	Onset—June 22 '46. Fever, severe lumbar pain, numbness in lower extremities; urinary retention, moderate degree of motor paralysis; positive Babinski; abdominal reflex—absent.	1st—26 Jun. '46 2nd—10 Jul. '46	0 159	AC Negative
47	M	F	Onset—June 17 '46. Fever, headache, dizziness, paralysis of left side and face. Positive Babinski—bilateral. Abdominal reflex, absent. Spinal fluid—30 cells; 92% lymphocytes.	1st—1 Jul. '46 2nd—9 Jul. '46 3rd—22 Jul. '46	0 1000 N. T.	N. T. 3+ (1:16) 3+ (1:8)
22	M	F	Onset—About Aug. 22 '46, marked weakness, fever; has been having headache, chill and chilly sensation. Liver, palpable; impression—malaria.	1st—26 Aug. '46 2nd—13 Sep. '46	27 369	2+ (1:2) 1+ (1:2)
30	M	F	Onset—about July 14 '46, with headache and joint pains. Paralysis of both upper and lower extremities with jaundice and headache, malaise, anorexia, nausea and vomiting.	1st—3 Aug. '46 2nd—21 Aug. '46 3rd—23 Oct. '46	159 1590 5630	3+ (1:16) 3+ (1:16) N. T.
21	M	F	Onset—July 27 '46. Fever, lethargy, headache, stupor, unconscious on admission. Foggy mental condition. Kernig and Babinski positive. RBC—4,500,000; WBC—10,650.	1st—3 Aug. '46 2nd—21 Aug. '46	3 7	Negative Negative

TABLE III—Continued

AGE yrs †	SEX	RACE	CLINICAL MANIFESTATIONS	DATE OF SPECIMEN	NEU- TRALIZA- TION INDEX	COMPLEMENT FIXATION TITER
22	M	W†	Onset—Aug. 13 '46, headache, general malaise, fever, chills and vomiting. Became delirious with intermittent periods of semi-stupor. Incontinence, rigidity of thigh muscles.	1st—20 Aug. '46 2nd—25 Aug. '46	5 25	Negative Negative
25	F	W	Onset—Oct. 9 '46. Severe headache, fever, photophobia and stiffness of neck. Irrational for short period. Lethargic. Positive Babinski. WBC—10,700.	1st—12 Oct. '46 2nd—23 Oct. '46	10 10	N.T. N.T.
20	M	F	Onset—About Dec. 20 '46. Pain in knee joints, followed with malaise and weakness of lower extremities. Pain became severe and muscles so weak that walking was impossible. Nausea jaundice. Afebrile except on first day of hospitalization, Jan. 3 '47.	1st—11 Feb. '47 2nd—23 Feb. '47 3rd—4 Mar. '47	252 3170 3170	N.T. N.T. N.T.
23	F	W	Onset—About Jan. 3 '47. Cough, fever and generalized weakness. Later, severe headache with photophobia. Spinal tap pressure—290 mm; normal cell and protein. RBC—4,490,000; WBC—7,000.	1st—9 Jan. '47 2nd—10 Feb. '47	10 10	N.T. N.T.
		W	No data available.	1st—16 Dec. '46 2nd—20 Dec. '46	10 10	N.T. N.T.
32	M	W	Onset—Jan. 5 '47. Headache, malaise, chills and fever. Later nausea, vomiting and marked weakness. Nervousness, choreic movements, ataxia; later—afebrile except for occasional rise to 99.8°F. Malarial smears—negative.	1st—22 Jan. '47 2nd—24 Feb. '47	31 10	N.T. N.T.

* Not tested.

† Filipino.

‡ White.

that these represent a good sampling of the population. Those positive in the Japanese group gave much higher neutralization indices than those in the Filipino group. This would make any speculation that these Japanese developed the antibodies as a result of exposure to the virus in the Philippines highly improbable.

TABLE IV
Neutralization tests on Philippine cattle

SPECIMEN	AGE	SOURCE*	NEUTRALIZATION INDEX
	<i>years</i>		
C-1	4	Batangas	3170
C-2	1½	Batangas	50
C-3	1½	Batangas	10
C-4	1½	Batangas	2000
C-5	2	Tayabas	10
C-7	5	Tayabas	10
C-9	4	Tayabas	200
C-10	6	Tayabas	10
C-11	4	Tayabas	3
C-12	5	Tayabas	126
C-13	4	Tayabas	126
C-14	5	Tayabas	10
C-16		Tayabas	10000
C-17	6	Tayabas	2000
C-18	5	Tayabas	10
C-21	5	Tayabas	63
C-22	6	Tayabas	1590
C-24	5	Tayabas	10
C-26	4	Tayabas	10000
C-30	4	Tayabas	10
C-31	5	Tayabas	10
C-32	5	Tayabas	3
C-32R	5	Tayabas	31
C-37	6	Tayabas	3
C-39R	4	Tayabas	10
C-42R	3½	Zamboanga	317
C-43	4	Sulu	10
C-44	5	Sulu	10
C-45	7	Sulu	159

* Provinces.

TABLE V
Neutralization tests on Philippine carabaos

SPECIMEN	AGE	SOURCE*	NEUTRALIZATION INDEX
	<i>years</i>		
Var-3.....	5	Mindoro	10,000
Car-6.....	2	Iloilo	3,170
Car-10R.....	2	Masbate	100
Car-15.....	4	Masbate	3,170
Car-18.....	4	Iloilo	10
Car-23.....	6	Romblon	10,000
Car-28.....	2	Masbate	100
Car-31.....	2	Mindoro	3,170
Car-34.....	2	Iloilo	79
Car-36.....	Unknown	Iloilo	1,000
Car-41.....	Unknown	Batangas	317

* Provinces.

In view of the absence of Japanese B encephalitis or encephalitis-like recent infection or vaccination among the Japanese and the length of time they have

TABLE VI
Neutralization tests on Philippine goats

SPECIMEN	AGE	SOURCE	NEUTRALIZATION INDEX
G-1	About	Alabang, Rizal	10
G-2	6	Alabang, Rizal	10
G-3	to	Alabang, Rizal	10
G-4	8	Alabang, Rizal	10
G-5	months	Alabang, Rizal	10
G-6	Unknown	Alabang, Rizal	10
G-7	About	Alabang, Rizal	100
G-8	1 year	Alabang, Rizal	3,170

TABLE VII
Neutralization tests on Philippine horses

SPECIMEN	AGE	SEX	SOURCE	NEUTRALIZATION INDEX
	<i>years</i>			
H-1			Manila	10,000
H-6	9	F	Lingayen, Pangasinan	7,950
H-7	1 $\frac{1}{4}$	F	Lingayen, Pangasinan	2,000
H-8	1 $\frac{1}{4}$	F	Lingayen, Pangasinan	100
H-9	7	M	Neuva Ecija	2,520
H-10	3	M	Batangas	1,260
H-11	2	M	Batangas	1,520
H-12	6	F	Australian, arrived in the P.I. 1947	7,950
H-13	4	M	?	1,520
H-14	7	M	Nueva Ecija	7,950
H-18	5	F	Masbate	3,170
H-19	2	M	Masbate	2,520
H-25	3	F	Masbate	3,990
H-26	3	F	Masbate	1,260
H-27	3	F	Masbate	12,600
H-28	3	F	Pangasinan	6,310
H-30	2	F	Pangasinan	2,000
H-31	2 $\frac{1}{2}$	M	Pangasinan	502
H-33	2	M	San Fernando, Pampanga	1
H-35	1 $\frac{1}{2}$	M	San Fernando, Pampanga	2,000
H-36	2	M	San Fernando, Pampanga	20,000
H-38	4	M	San Fernando, Pampanga	1,590
H-43	3	F	Nueva Ecija	50

been away from Japan, it would appear that the neutralizing as well as complement fixing antibodies persisted at least two to four years.

In the suspected human encephalitis cases (Table III) 5 of the 13 gave a very

definite rise of titer in neutralizing antibodies. On the assumption that Japanese B virus is not present in the Philippines it is difficult to explain the rise in titer unless other unknown diseases will stimulate such reactions.

In the animal groups particularly the carabaos (Table V) horses (Table VII) and cattle (Table IV) there are indices of such a high degree that it is very doubtful if such could be stimulated by other infections.

Cattle, rats, guinea pigs, dogs, rabbits and birds have been claimed to be completely insusceptible to the encephalitic complication, yet their blood may show detectable amounts of virus and varying amounts of antibody (8). It is possible the virus may affect carabaos and goats in the same manner.

SUMMARY

1. Neutralization tests on 21 Filipino civilians for Japanese B encephalitis showed 7 with positive indices or 33.3 per cent.

2. Fourteen of the 17 Japanese prisoners of war whose sera were tested gave positive indices or 82 per cent. These indices were higher than those in the Filipino group.

3. Fourteen of 29 cattle gave positive indices or 45 per cent; 21 of 23 horses or 91 per cent; 10 of 11 carabaos or 90 per cent and 2 of 8 goats or 25 per cent gave positive neutralization indices.

4. Serial specimens from 13 patients suspicious for Japanese B encephalitis virus infection were obtained and tested. Five of these serial specimens showed a tenfold or greater increase of neutralizing antibodies.

CONCLUSION

Neutralizing antibodies for Japanese B encephalitis virus of significant titers have been found among suspected human cases, and certain Philippine animals (cattle, horses, carabaos and goats).

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PREPARATION AND TESTING OF A SPECIFIC ANTIGEN FOR DIAGNOSIS OF HUMAN STRONGYLOIDIASIS¹

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The literature contains only two papers dealing directly with the immunological aspects of strongyloidiasis. These are by Sato (1) on agglutination of filariform larvae by immune sera, and by Fülleborn (2) on a scratch test for strongyloidiasis. Using the serum from an animal infected with *Strongyloides*, Sato observed that the homologous infective filariform larvae formed clumps in a suspension which was mixed with the serum.

The work done by Fülleborn (2) is of particular interest with reference to the present study. This investigator collected filariform larvae of *Strongyloides stercoralis* from fecal cultures, washed them in 1:1000 bichloride of mercury solution and allowed them to dry at room temperature. He then ground them up in an agate or porcelain mortar. The powder, stored in well-stoppered glass bottles, remained active for one year, and was used in scratch testing on the forearm. Of 11 cases tested (10 with positive stools and the eleventh with a suggestively positive stool), he obtained 4 one-plus reactions and 7 two-plus and three-plus reactions. He expressed the belief that positive reactions remained for sometime after the patient has become parasite-free. Twenty-eight cases, which were not infected with *Strongyloides* but many of which had *Ascaris* or had been cured of ascariasis, were also tested and all were negative to the *Strongyloides* antigen. Fülleborn (*l.c.*) concluded that a positive reaction with a scratch test for strongyloidiasis indicates a present or a past infection with this helminth. He felt it to be of diagnostic value, although it was not known how soon after infection a positive reaction would appear or how long it would remain. He reported that one positive reaction was obtained one and one-half years after the infection had apparently been terminated. He expressed the belief that adults were as effective as larvae for preparing this antigen.

THE PROBLEM

The investigation herein reported has been concerned with (1) the preparation of a *Strongyloides* antigen for use in quantitative tests and (2) an investigation of immunological reactions in human strongyloidiasis.

MATERIALS AND METHODS

On undertaking this problem the first consideration was an adequate source of material from which to prepare an antigen. The several stages in the life

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cycle of *Strongyloides* which might provide the antigenic substances are: free-living adults, rhabditoid larvae, filariform larvae and parasitic adults. Free-living adults are not readily obtainable in most human infections with *Strongyloides stercoralis*, being found only in cases presenting the indirect type of development. Furthermore, they are not satisfactory for the preparation of pure antigen because it would contain a large amount of contaminative antigenic substance from ingested bacteria. Rhabditoid larvae, also feeders on bacteria, are excluded for the same reason. The parasitic adults (females) are difficult to obtain since they are buried in the intestinal mucosa throughout their adult life. The filariform larvae are non-feeders and can be secured in a relatively bacteria-free condition in the moisture of condensation as it occurs in ordinary petri-dish cultures of fecal material. It was readily apparent that this stage was the most suitable source of material.

To obtain a constant supply of filariform larvae, it was necessary to seek an animal source of *Strongyloides*. Chimpanzees were thought to be the most satisfactory since their strain of *Strongyloides* (presumably *Strongyloides fülleborni*) is usually an indirect type and yields a large number of filariform larvae from the free-living generation. Two chimpanzees (*Pan satyrus*) in the Audubon Park Zoo, New Orleans, both with heavy infections, were available for a short period of time. Fecal specimens were collected daily and placed in culture with animal charcoal powder. The filariform larvae appeared in the moisture of condensation, beginning on the fourth day of culture in the case of one chimpanzee and on the eighth day in the other. For a period of four days they were present in large numbers but thereafter the larval yield was too small to justify saving the cultures. To insure the filariform larvae egress to the moisture of condensation, it was necessary to moisten the petri dish lid daily, thus maintaining an unbroken water film in which the larvae could migrate to the top.

These larvae are strongly phototactic. This characteristic was utilized in collecting them. The petri dish lid was removed and moistened. The dish was then placed in position with a bright artificial light shining directly down on it for a period of 30 to 40 minutes. The living larvae immediately responded by swarming up the sides of the dish. This technic was employed once a day during the scantier productive days and several times daily during "swarm" days. Use of light in collecting the larvae helped to insure that the forms collected were living. It was felt that the chemical changes following death of the larvae and subsequent bacterial invasion might introduce unknown foreign antigenic substances into the extracted material.

At the end of the periods of exposure of the cultures to light the lid was removed, quickly inverted and washed with 0.425 per cent salt solution (approximately isotonic for nematodes) containing 0.5 per cent phenol. The lid was then tilted back and forth so that the solution was washed down the entire surface. The harvest was then pipetted into a 40 cc. test tube and stored in the refrigerator. The larval yield at the end of each three weeks amounted to one to three cc. of packed organisms and was considered adequate for extraction studies.

With practice it was found that the worms could be separated by suspending them in the isotonic salt solution and adjusting the speed of the centrifuge. At less than 500 revolutions per minute for two minutes any adults which might have migrated into the water of condensation were thrown to the bottom of the tube and removed by means of a capillary pipette. Unbroken filariform larvae were thrown down on centrifugalization at approximately 1200 r.p.m. for the same length of time, leaving rhabditoid larvae and broken forms in the supernatant fluid, which was poured off. Repetition of the process 4 to 5 times insured a residue of whole filariform larvae. These were resuspended in sterile isotonic salt solution containing 0.5 per cent phenol and spun in the centrifuge at 1500 r.p.m. for 2 minutes. The supernatant fluid was discarded and the process repeated 5 to 8 times, after which the larvae were transferred to a clean, sterile test tube which was closed with a clean, sterile cork stopper. Washing was continued in this tube 10 to 12 times before transferring to a third sterile test tube with a sterile cork. The material was then cultured for sterility by inoculation into brain broth. As soon as sterility was established, the larvae were ready for use in preparation of an antigen. They were stored in the refrigerator when they were not being processed.

PREPARATION OF ANTIGEN

Several methods for the preparation of antigen were employed, two of which produced active testing material. Only these two will be described.

Strongyloides antigen I. Sterile larvae were placed in a steel-ball mill which had been sterilized. To this were added sterile steel balls and sterile isotonic salt solution. The ball mill was set in motion and allowed to run for 24 hours. The crushed larvae were transferred to a sterile flask, the ball mill was flushed out with 50 cc. of sterile isotonic salt solution and this effluent added to the flask. The flask was kept at 37°C. for 72 hours with frequent shaking during that interval. The material was then placed in a large petri dish and left open at room temperature to allow dehydration to occur. When the volume had been reduced to one-third the original amount, the extract was put through a Seitz filter, bottled in a brown glass bottle, tested for sterility and stored in the refrigerator. This proved to be an active but not a very potent antigen.

Preliminary attempts showed that a positive antigen of good potency could be produced by grinding the larvae with emery powder in an agate mortar and extracting in Coca's solution. After the value of this method was established, this method was employed to prepare an antigen for quantitative studies.

Strongyloides antigen II. Sterile powdered emery powder was placed in a sterile agate mortar. The grinding took place under a metal hood which was designed for virus transfer work. The hood was flamed on the interior to insure sterility. The table on which it was placed and the front opening of the hood were draped with sterile linen and the worker wore sterile gloves during the grinding.

Sterile larvae were suspended in Coca's solution (0.5 per cent NaCl, 0.5 per cent Na_2HCO_3 and 0.4 per cent phenol) and pipetted into the mortar. The

volume of the suspension was 3.9 ml. From the number of intact larvae counted in 0.025 ml. of the suspension, the total number of larvae was calculated to be about 4 million. The larvae were ground for one and one-half hours at room temperature by manual manipulation. The mixture was then washed into a flask containing glass beads. Extraction continued at room temperature for 24 hours and at 37°C. for 24 hours. The antigen solution was put through a Seitz filter and placed in a sterile, chemically clean, weighed crucible. It was transferred to a desiccator and dehydration was carried out over sulfuric acid under vacuum. The completely dried residue was weighed, returned to the desiccator for another 24 hours and reweighed. Since no additional loss of weight occurred, the antigen was considered completely dried. This weight was taken as a basis for calculation. The antigen was then put back into solution with 25 ml. sterile distilled water, bottled and tested for sterility.

This quantitative procedure was checked using 2,500,000 larvae. Calculation showed that the two extractions had produced comparable results. The dried weight of the first preparation (352 mgm.) minus the dried weight of a corresponding amount of Coca's solution (109 mgm.) was 243 mgm., while the second preparation gave a net dried weight of 149 mgm. The powdered residue from each of these two preparations was dissolved in sterile distilled water to make a dilution of 1:100. The two solutions were then pooled and constituted the stock solution of *Strongyloides* antigen for quantitative tests. The total stock antigen consisted of 39.2 ml. Standard tests for contamination demonstrated that the solution was bacteriologically sterile.

Hookworm antigen. Attempts to secure an adequate number of filariform larvae of *Ancylostoma caninum* were unsuccessful. Two hundred thirty living adults of this species were obtained from autopsied animals, washed free of bacteria and extracted as for *Strongyloides* antigen II. The dried residue minus the weight of the Coca's solution was 60 mgm. This material was made up in a 1:100 dilution with sterile distilled water and used as control antigen.

Bacterial antigen. A suspension of bacteria from fresh chimpanzee fecal material was made in physiologic salt solution and this was inoculated into brain broth. Transfers were made to blood agar and beef broth. Bacteria were collected from these cultures, prepared by crushing in the steel-ball mill and extracting in Coca's solution at 37°C. for 48 hours. The extract was then passed through a Seitz filter. When sterility of the finished antigen was demonstrated, this antigen was made up in a 1:100 dilution and was used as a control. No attempt was made to identify the species of bacteria, as very little information is available concerning the intestinal flora of the chimpanzee.

Chemical analysis of the antigens was undertaken on a small amount of material remaining after the immunological tests were completed.

Nesslerization:

Antigen I.—1 ml.—.032 mgm. N.

Antigen II.—1 ml.—.0366 mgm. N.

Hookworm Antigen.—1 ml.—.032 mgm. N.

Molisch Test: negative

Biuret Test: negative

Millon's Test: negative

Thermal tests: The antigen was sealed in ampules and subjected to temperatures of 56°, 70°, and 100°C. for 40 minutes. Other ampules were autoclaved for 20 minutes at 15 pounds pressure. Thermally-treated antigen samples were used for intradermal tests to determine the effects of heating on the potency of the antigen.

The standard tests for protein and carbohydrate were negative, but nitrogen was shown to be present by Nesslerization. Thus, one must assume that it was present either as an amino acid or as a protein combination which prevented the usual protein reaction from occurring with the Biuret test.

THE IMMUNOLOGICAL TESTS EMPLOYED

The intradermal test. The area on the volar surface of the forearm to be tested was cleaned with 70 per cent alcohol. The amount of fluid injected, antigens and controls alike, was 0.1 cc. Patients were first tested with Coca's solution. If a positive reaction occurred with this solution the patient was not used in the study. All test materials contained phenol and patients who reacted to the phenol in the Coca's solution had a skin which was too sensitive to give accurate intradermal readings. Patients with dermatographia were not tested for the same reason. If the patient did not react to Coca's solution, the tests were then done on the patient, the Coca's solution injection being repeated as control at the time of the other tests.

Readings of the intradermal reactions were made in 15 minutes and were continued until disappearance of the reaction. The earlier tests were also read at 24 and 48 hours but were consistently negative. The reactions were therefore found to be of the immediate type. A wheal, frequently with pseudopods, appeared in 5 to 7 minutes and was soon followed by an encircling erythema. The reaction reached its height in 10 to 15 minutes, began to fade in 20 minutes and had completely disappeared within two hours. In interpreting intradermal reactions, a wheal of 3 cm. diameter with pseudopods projecting into the surrounding zone of erythema was read as ++++. A wheal of 2.5 to 3 cm. diameter without pseudopods but with surrounding erythema was rated ++++. A wheal of 2 to 2.5 cm. diameter with erythema, with or without pseudopods, was classified as ++. A diameter of 1.5 to 2 cm. with erythema, and with or without pseudopods, was rated +.

The precipitin test. The tubes used for the precipitin tests were 50 mm. in length and had a 5 mm. inside diameter. The undiluted serum to be tested was placed in the tube by means of a finely drawn capillary pipette. The tip of the pipette was introduced to the bottom of the tube and 0.5 ml. of serum allowed to enter the tube. The serum was then overlaid with 0.5 ml. of each of the several antigenic dilutions (*Strongyloides* antigen II). The diluent used in making the antigenic dilutions was sterile physiologic salt solution. The pH of the antigen used was adjusted to 7.0 from its normal 7.2. This was done to eliminate any chemical factor altering the precipitin reaction. Controls were run on the diluent, the serum, and the bacterial antigen. The series of dilutions used was as follows: 1:1,000, 1:2,000, 1:5,000, 1:10,000, 1:15,000, 1:20,000, 1:30,000, 1:50,000, 1:100,000, 1:500,000, 1:1,000,000. Readings were made

TABLE I
Laboratory data on *Strongyloides patients*

CASE NO.	CON- TROL (COCA'S SOL.)	DACT. ANT.	HIV. ANT.	INTRA- DERMAL TEST (ANT. I)	INTRADERMAL TEST (ANT II)	PRECIPITIN TITER	EOS. RISE	FECAL EXAM. PER AV. DIRECT FILM	FECAL EXAM. PER AV. CONC. FILM	HB% (DAVE)	RBC	TOTAL WBC	PMN (YOUNG)	PMN (MA- TURE)	LYMPH	MONO.	EOS.	BASO.
Group A. Patients infected with <i>Strongyloides</i> at the time of the tests																		
1	-	-	-	++	++	1:20,000	1	1rh	1rh	76	3.25	10,800	59	8	26	6	1	1
2	-	-	-	++	++	1:20,000	3	1rh, 1f	1rh	86	4.1	13,600	43	8	32	8	8	1
3	-	-	-	++	++	1:15,000	4	1rh, 4f	4rh,	75	5.5	13,100	45	1	29	4	21	7
4	-	-	-	++	++	1:15,000	2	3f	3f	80	5.6	12,800	53	3	30	6	7	8
5	-	-	-	++	++	1:20,000	1	12f	3rh,	75	5.2	7,700	64	3	29	3	8	1
6	-	-	-	++	++	1:20,000	3	24rh	24rh	68	5.4	12,000	52	6	34	3	6	1
7	-	-	-	++	++	1:20,000	4	58f	2rh,	77	5.3	12,000	50	10	30	4	5	1
8	-	-	-	++	++	1:30,000	4	2f	3rh,	85	4.9	15,500	37	16	57	2	7	1
9	-	-	-	++	++	1:15,000	4	1f	3rh,	79	3.9	9,700	34	1	51	1	12	1
10	-	-	-	++	++	1:20,000	4	18f	6rh,	75	4.6	9,500	29	2	43	1	25	1
11	-	-	-	++	++	1:30,000	1	38rh,	3rh,	65	3.2	9,000	53	1	43	2	2	1
12	-	-	-	++	++	1:20,000	2	2f	28rh,	60	3.8	10,100	39	2	50	1	9	1
13	-	-	-	++	++	1:20,000	1	48rh,	2f	81	4.8	13,100	57	2	33	1	6	1
14	-	-	-	++	++	1:10,000	1	1f	10f	80	5.2	16,800	62	2	29	2	4	1
15	-	-	-	++	++	1:20,000	2	52rh,	7f	77	5.1	8,500	52	2	34	4	7	1
16	-	-	-	++	++	1:15,000	1	1rh	52rh,	73	3.6	11,400	56	1	31	1	10	1
17	-	-	-	++	++	1:30,000	1	1rh	1rh	85	6.0	14,250	50	1	36	2	10	1
18	-	-	-	++	++	1:5,000	1	8f	8f	73	3.3	16,800	89	3	7	1	4	1
19	-	-	-	++	++	1:30,000	2	2rh, 1f	5rh,	88	4.65	8,000	44	3	48	4	7	1
20	-	-	-	++	++	1:20,000	3	2rh	1rh,	88	5.4	8,400	52	2	35	1	5	1
21	-	-	-	++	++	1:30,000	3	1rh	14rh,	79	4.6	10,500	72	1	21	1	19	1
22	-	-	-	++	++	1:20,000	2	3rh	21rh	63	7.3	15,300	51	2	27	2	1	1
23	-	-	-	++	++	1:15,000	2	9rh,	9rh,	80	4.5	17,800	67	2	29	2	8	1
24	-	-	-	++	++	1:20,000	2	6rh,	6rh,	77	4.5	13,800	63	2	27	1	9	1
25	-	-	-	++	++	1:20,000	2	1rh, 6f	20rh,	85	5.7	9,500	69	1	30	1	9	1
Group B. Individuals who previously harbored <i>Strongyloides</i>																		
1	-	-	++	±	±	neg.	1			73	5.0	10,000	44	10	33	1	11	1
2	-	-	++	±	±	1:15,000				82	4.3	11,000	55	3	24		18	
3	-	-	++	±	±	1:1,000				76	4.9	8,500	45	2	49	2	2	
4	-	-	++	±	±	1:30,000				75	4.9	7,250	28	1	47		19	2

rh—rhabditoid larvae, f—filariform larvae of *Strongyloides*.

after the test had remained at room temperature for one hour. The rack was then placed in the refrigerator and a reading was made in 5 to 6 hours. The

TABLE II

Synopsis of positive intradermal reactions among 108 controls without evidence of Strongyloides infection (Group C)

INFECTION	NO. OF CASES	BACTERIAL ANTIGEN	STRONGYLOIDES ANTIGEN	HOOKWORM ANTIGEN
Negative.....	2	+	+	+
Enterobius.....	1	±	±	±
Ascaris & Trichocephalus.....	1	±	+	±
Trichocephalus.....	1	±	±	±
Hookworm.....	1	—	—	+++++
	1	—	±	+++++
	1	—	—	+++
	2	—	±	+++
Hookworm & Trichocephalus.....	1	—	—	++
	1	—	±	+++
Hookworm & Enterobius.....	1	—	++	+++
Ascaris.....	1	—	±	+++
	1	—	±	++
	1	—	+	++
	1	—	—	+
	2	—	—	±
	(6)	—	—	—
Ascaris & Trichocephalus.....	1	—	+	+
	1	—	±	+
	(2)	—	—	—
Ascaris & Enterobius.....	1	—	+	+++
Trichocephalus.....	1	—	—	±
Trichocephalus & Enterobius.....	(1)	—	—	—
Enterobius.....	1	—	+	+
	1	—	+	+
	1	—	—	+
Helminth (unspecified).....	(2)	—	—	—
	1	—	+	+++
	1	—	+++ (ppt. neg.)	—
No demonstrated helminths.....	1	—	++	+++
	2	—	+	+
	1	—	±	±
	2	—	±	—
	4	—	—	++
	2	—	—	±
Total cases with positive reactions...	1	—	—	+
	41			

degree of white-ring formation at the interphase was the basis for reading the reactions. The lowest dilution at which an interphase ring developed was recorded as the precipitin titer.

RESULTS

The patients tested in this study may be divided into 3 groups:

A. Twenty-five patients, proved by stool examination to have strongyloidiasis at the time the tests were made. All of these received tests with Antigen II as well as control tests with the bacterial antigen and Coca's solution. Antigen I was also used on patients 4 through 25 and hookworm antigen on patients 15 through 25.

B. Four patients who previously harbored *S. stercoralis*. These patients were tested with Antigen II, Antigen I, Coca's solution, hookworm antigen, and bacterial control antigen.

C. One hundred and eight patients negative for *S. stercoralis* by fecal examinations at the time the tests were performed and without a previous history of the infection. These individuals were tested with Antigen II, hookworm antigen, control bacterial antigen and Coca's solution control.

The dilution of the *Strongyloides* and hookworm antigens used was 1:100. The control antigen was a Coca's solution extraction of an equivalent volume of bacteria obtained from culture of the chimpanzee stool specimens.

All differential white blood counts were made by counting 200 cells.

As a matter of comparative interest in the immunological reactions total red and white blood counts, Hb estimation and differential white counts were made on all *Strongyloides* patients.

Summary data are presented in tables I and II.

DISCUSSION

The value of this study is contingent on four main factors, viz., (1) specificity and potency of the *Strongyloides* antigen, (2) selections of the patients on whom the tests were made, (3) care with which the tests and the control tests were performed and read and (4) diagnostic significance of the tests.

The antigen. The source material of filariform larvae from cultured feces of the chimpanzee was clean and relatively abundant. The antigen was processed according to standard technics for preparation of helminth antigen. It was demonstrated to be bacteria-free and the only known contaminant was phenol in isotonic salt solution in which the filariform larvae were first suspended and in the Coca's solution in which the larvae were placed during their maceration and the extraction of the antigenic fractions. Antigen I was not quantitated but Antigen II was carefully weighed and measured amounts were employed in both the intradermal and precipitin tests, as were also the bacterial and hookworm antigens in the control tests.

Selection of patients. All patients in the series designated as Group A were demonstrated to be infected with *Strongyloides stercoralis* at the time of the tests by stool examination, either by direct film or zinc sulfate centrifugal floatation concentration. The history of these individuals, together with the blood studies and the sparseness of *Strongyloides* larvae in the stools of all but a few (see table I), are consistent with a diagnosis of chronic strongyloidiasis. None of the pa-

tients had positive Kline or Kolmer reactions. Case 2 had a previous record of hookworm and *Enterobius* infections; cases 14, 16 and 24 had concurrent mild whipworm infection, and case 21 had a light hookworm infection. Several had intestinal protozoa, including cases 13, 23 and 24 with demonstrated amebic colitis. This group of 25 persons constituted a relatively homogeneous one and was considered satisfactory for analysis.

Group B (table I) was too small for comparison with Group A but provided some useful information. No. 4 of this group is the record of the senior investigator, who early in the study became accidentally infected, was successfully treated but remained highly sensitive to *Strongyloides* antigen, as the tests revealed.

Group C (table II), consisting of 108 persons without demonstrable evidence of strongyloidiasis but with a considerable number of other helminthiasis, represented a cross section of the average clinic population in New Orleans and served as a control for Group A.

Performance and reading of the tests. It is unnecessary to repeat the details described above for carrying out the intradermal and precipitin tests (*vide supra* under "The Immunological Tests Employed"). It should be emphasized, however, that they were carried out according to standard methods. In order that reactions to nonspecific antigens might be detected all groups were also tested intradermally with Coca's solution, bacterial antigen prepared from the feces of the chimpanzee and all except the first 14 of Group A with hookworm antigen prepared from the dog hookworm (*Ancylostoma caninum*).

Diagnostic significance of the tests. In Group A, 23 of the 25 cases demonstrated definitely positive reactions to *Strongyloides* antigen, both by the intradermal and precipitin tests, and none of the 25 reacted to Coca's solution or bacterial antigen, while only three (16, 19 and 20) of the eleven tested with hookworm antigen gave a questionable positive test. The high dilution of the antigen in the precipitin test, which demonstrated antibodies in all patients except No. 18, suggests *active reaction* to the antigen. Case 18 gave a doubtful reaction to the intradermal test. At the time the test was performed he was moribund and had possibly lost his ability to produce antibody in expected amounts. He was apparently approaching the anallergic state when skin reactions are lost. Case 22, also with a rather questionable intradermal reaction but with a low-titer precipitin test and high eosinophilia, was suffering from severe exfoliative dermatitis.

In Group B, individuals 1 and 3 developed doubtfully specific reactions to *Strongyloides* antigen, suggesting that their previous infection had been eliminated. On the other hand, the immunological evidence in tests with individuals 2 and 4 probably indicates residual infection or at least active antibody formation.

In Group C (table II), the first five cases listed provided evidence of mild sensitivity to all three antigens tested intradermally. Twenty-seven other cases in this series, 19 with demonstrable helminthiasis, provided 23 definite and four questionable intradermal reactions to hookworm antigen, while 10 were positive and 8 others questionable in reaction to *Strongyloides* antigen. In eight instances

of patent hookworm infection the tests with hookworm antigen were strongly positive (++ to ++++); one of these (hookworm with *Enterobius*) also provided a 2-plus reaction with *Strongyloides* antigen. The specificity of the hookworm reaction in the hookworm infections suggests that the positive reaction to this antigen in the *Ascaris* and *Enterobius* infections may also be diagnostic of hookworms undiagnosed by stool examination, while there is suggestive evidence that "Helminth (unspecified)" is also a hookworm. Similarly, under "No demonstrated helminths" there are one case having a 3-plus test and four cases having 2-plus intradermal tests with hookworm antigen in which hookworms conceivably might have been present but were undiagnosed in the stool. In one of these there was also a 2-plus reaction to *Strongyloides* antigen, while there was an additional instance in which the *Strongyloides* intradermal test was 3-plus, although a precipitin test on this patient was negative.

By way of summary, it may be stated that the specificity of the *Strongyloides* antigen employed in this study was demonstrated in 23 of 25 patients found by stool examination to harbor *S. stercoralis*, while the two questionable reactions are explained by the clinical condition of the patients. In the 108 control subjects two reactions of 2-plus occurred when *Strongyloides* antigen was tested. These may possibly be interpreted as residual sensitivity from former *Strongyloides* infection. The single 3-plus intradermal reaction with *Strongyloides* antigen was not substantiated by the precipitin test. Additionally, there were two one-plus reactions to *Strongyloides* antigen in persons shown to be sensitive also to bacterial and hookworm antigens, and seven additional one-plus readings in the control series. If 2-plus reaction is taken as the minimal critical level of definite reaction, there were three positive intradermal reactions with *Strongyloides* antigen in the 108 controls which were not correlated with demonstration of *S. stercoralis* in the stools.

Considering the data presented in table I (Group A, *Strongyloides*-positive cases) and table II (Group C, in which *Strongyloides* was not demonstrated in the stools), it is relevant to inquire if there is adequate evidence that *Strongyloides* antigen, as prepared and employed in this investigation, is diagnostically specific. Statistically, comparing 23 out of 25 positive tests in *Strongyloides*-positive persons with three definitely positive tests (2-plus or more) in 108 persons without evidence of *Strongyloides stercoralis* in their stools, it is improbable ($P < .001$) that the high correlation in Group A would occur by chance alone. It may be stated, therefore, with considerable assurance that the test as performed was highly specific for strongyloidiasis.

SUMMARY AND CONCLUSIONS

A test antigen prepared from clean filariform larve of *Strongyloides* cultured from the feces of the chimpanzee and extracted in isotonic (0.425 per cent) salt solution or Coca's solution, in dilution 1:100 produced 23 positive intradermal reactions and 25 positive precipitin tests in 25 chronic cases of human strongyloidiasis. Additionally there were two doubtful intradermal reactions, one in a moribund patient and one in a patient who had very severe exfoliative dermatitis.

The precipitin titers ranged from 1:5,000 to 1:30,000. This antigen produced consistently negative, doubtful or weak intradermal and precipitin tests in 105 of 108 presumably uninfected control cases.

The diagnostic significance of the positive intradermal and precipitin reactions has been demonstrated in these cases of *Strongyloides* infection. The tests provide a reliable index of infection. In cases of presumably cured *Strongyloides* infection the value of the intradermal and precipitin test is not so clear. It is not known how long after cure the intradermal reaction will remain positive, although it is likely that the precipitin test as carried out indicates the presence of specific antibodies.

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ACTION OF SOME ALKYLHYDROXYBENZENES ON PIG ASCARIS IN VITRO

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An extensive and thorough study of the anthelmintic properties of alkylhydroxybenzenes has been made by Lamson and his associates (1-4). These authors systematically tested more than 150 different compounds and initiated the therapeutic use of hexylresorcinol in certain helminth infestations. It has been shown (5) that the introduction of halogen into hydroxybenzenes enhances their antiseptic potency. Hartman and Schelling (6) have previously studied a number of new halogen-substituted hydroxybenzenes for their antiseptic properties. Among those substances, there were some with very high germicidal activity. It therefore seemed of interest to test these compounds also for their anthelmintic properties.

METHODS

Tests were run according to the *in vitro* method of Lamson and Brown (7). Briefly, this method consists of exposing well-washed worms to the drug in 1:1000 saline suspension for varying periods of time with constant stirring. After keeping the worms in physiological saline in the incubator overnight, they are then tested for motility by dropping them into water at 60° C. Those showing no motility are considered dead. The pig ascaris were obtained from the abattoir in warm 0.85 per cent saline in a large thermos jar. Upon arrival at the laboratory, they were thoroughly washed in warm water and stored in saline in the incubator at 37.5° C. until needed. Our initial exposure to the compound under investigation was carried out in a 400 cc. beaker on the water bath at 37.5° C. using a glass rod for hand stirring. Most of the compounds tested are less soluble than 1:1000 in saline at 37.5° C. By constantly stirring, the compound is kept in suspension.

A number of tests were also run by the kymographic method using the technic of Baldwin (8). A 3 cm. segment of a whole worm is taken at a point just anterior to the genital pore. It is attached to a muscle lever and immersed in a small chamber of saline in a water bath at 37.5° C. An emulsion of the drug to be tested is prepared by using a 0.05 per cent concentration of sodium glycocholate for stabilization. The drug emulsion may now be placed in the chamber and its action upon the worm segment recorded on the kymograph.

The compounds used were prepared by one of us (Schelling) by a method already described (6). Type formulae for these compounds are shown in Figure 1.

RESULTS

Table 1 shows the percentages of worms killed by the different compounds used. As a control substance, hexylresorcinol was employed. The results are

shown in the table and are in agreement with those of Lamson and Brown. From this table, it can be seen that one compound, 2-ethyl-4-chloro-6-hexylresorcinol, has a range of activity somewhat akin to that of hexylresorcinol. In

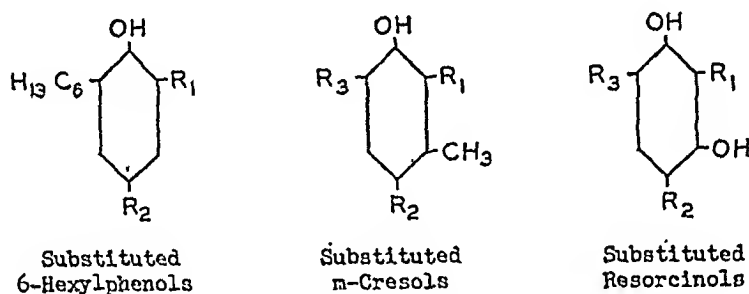


FIG. 1. TYPE FORMULAE FOR COMPOUNDS USED

TABLE 1

Percentage of pig ascaris killed by different lengths of exposure to a 1:1000 saline suspension of the drug at 37.5°C.

	TIME IN MINUTES							
	1	2	5	10	20	30	45	60
2,4-Dichloro-6-hexylphenol.....	0	0	0	0	0	0	0	0
4-Chloro-6-hexyl-m-cresol.....	0	0	0	0	50	100	100	100
2-Ethyl-4-hexyl-6-chloro-m-cresol.....	0	0	50	100	100	100	100	100
2,4-Dihexyl-6-chloro-m-cresol.....	0	0	0	50	100	100	100	100
2-Bromo-4-hexyl-6-chloro-m-cresol.....	0	0	0	0	50	50	50	100
Hexylresorcinol.....	50	100	100	100	100	100	100	100
Resorcinol-4-hexylketone.....	0	50	100	100	100	100	100	100
4,6-Dihexylresorcinol.....	50	100	100	100	100	100	100	100
4-Chloro-6-heptylresorcinol.....	0	50	100	100	100	100	100	100
2-Hexyl-4,6-dichlororesorcinol.....	0	0	0	0	25	25	100	100
2-Hexyl-4,6-dibromoresorcinol.....	0	0	0	100	100	100	100	100
2-Ethyl-4-chloro-6-hexylresorcinol.....	100	100	100	100	100	100	100	100
2-Hexyl-4-chloro-6-acetylresorcinol.....	0	0	0	0	0	0	0	0

TABLE 2

Percentage of pig ascaris killed by different lengths of exposure to a 1:5000 saline suspension of the drug at 37.5°C.

	TIME IN MINUTES			
	1	2	5	10
2-Ethyl-4-chloro-6-hexylresorcinol.....	43	100	100	100
Hexylresorcinol.....	0	8	56	100

order to evaluate these results more closely, we repeated the experiments using a 1:5000 suspension in saline. The results are shown in Table 2. In this concentration, hexylresorcinol becomes soluble, while 2-ethyl-4-chloro-6-hexylresorcinol remains partially insoluble.

Figure 2 shows a kymographic record of the action of a worm segment in a 1:10,000 suspension of 2-ethyl-4-chloro-6-hexylresorcinol using sodium glycocholate for stabilization. Experiments using 1:20,000 hexylresorcinol do not produce any action within 40 minutes. Baldwin has shown that hexylresorcinol produces paralysis of the worm within 20-30 minutes if used in concentrations of 1:10,000 to 1:5,000.

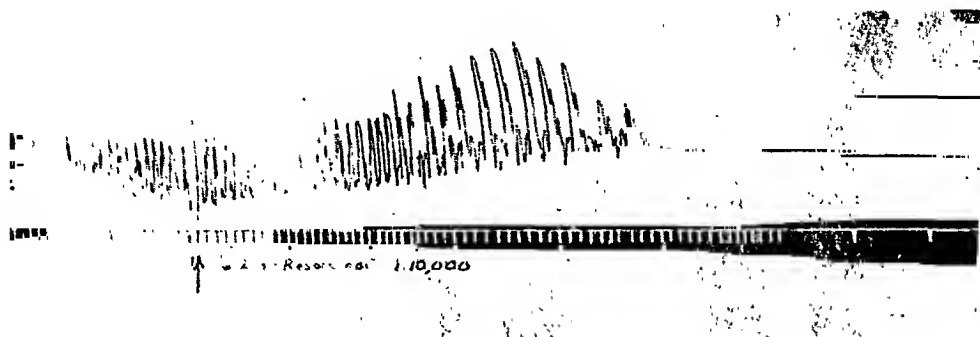


FIG. 2. ACTION ON A SEGMENT OF PIG ASCARIS AT 37.5°C. OF A 1:10,000 SUSPENSION OF 2-ETHYL-4-CHLORO-6-HEXYLRESORCINOL IN PHYSIOLOGICAL SALINE STABILIZED BY THE ADDITION OF 0.05% SODIUM GLYCOCHOLATE. TIME SHOWN IN 1-MINUTE INTERVALS.

TABLE 3

Oral toxicity of 2-ethyl-4-chloro-6-hexylresorcinol for rats. Denominator indicates number of rats used. Numerator indicates number of rats dying.

Dosage, cc./kilo	0.05	0.1	0.2	0.4	0.6	0.7	0.8	1.0	1.5	2.0
Mortality	0/1	0/1	0/1	2/4	1/4	1/1	2/3	2/4	1/2	1/1

TOXICITY

Preliminary toxicity studies on 2-ethyl-4-chloro-6-hexylresorcinol have been done on rats, the results of which are shown in Table 3. Although these results are by no means complete, they would seem to indicate that the range of toxicity is somewhat comparable to that of hexylresorcinol. Lamson and Brown (7) report the L.D. 50 of hexylresorcinol for rats as 0.35 gm./kg.

Like other resorcinols, 2-ethyl-4-chloro-6-hexylresorcinol is somewhat escarotic. A drop of the pure substance on the tip of the human tongue for 2 minutes produces a moderate whitened area which lasts for 24 hours and a burning, stinging sensation which lasts for about 6 hours and then gives way to the absence of sensation, much as is experienced from the drinking of hot liquids. A drop of the pure substance on the volar surface of the human forearm for a period of 5 minutes produces a mild stinging sensation with a reddened discoloration which becomes a thin, crusted area in about 24 hours and is completely healed after 4 to 5 days. No definite ulceration is ever present.

DISCUSSION

A number of difficulties are encountered in any comparison of anthelmintics by *in vitro* methods. Not the least of these is the fact that many of the com-

pounds to be tested are not soluble in water. The drugs must therefore be tested either in suspension or by the addition of an emulsifying agent. The results then become subject to the many variations which are inherent with emulsified solutions. Lamson and Brown, however, after a very extensive study, have deemed it advisable, for screening purposes, to use a saline suspension. We have followed their suggestion and have found it adequate for our purposes. Baldwin believes that the drugs should be tested in emulsified solutions, so that in following the kymograph method, we have used his directions in order that results might be comparable.

In their large series of compounds, Lamson and Brown report a number which are as effective as hexylresorcinol. These, they say, should be given a clinical trial after adequate toxicity and pharmacological studies. In their study of a group of halogenated phenols (3), they report two compounds especially which have high ascaricidal properties. Our studies tend to confirm their work in that the introduction of certain halogens tends to enhance the anthelmintic property of the compound.

Lamson and Brown were also seeking an anthelmintic with the excellent ascaricidal properties of hexylresorcinol but without the local irritation which that drug produces upon the oral mucus membranes. Our compounds seemed to fall within about the same general range in regard to irritant action as do the other resorcinols.

Compounds with high anthelmintic properties and low toxicity and irritant properties also deserve a clinical trial for the additional reason that they might be effective against infestations by other parasites such as necator or trichuria.

Further studies on the toxicology of 2-ethyl-4-chloro-6-hexylresorcinol will be conducted and should these permit, a clinical trial is anticipated.

CONCLUSIONS

Several new substituted alkylhydroxybenzenes have been tested for their ascaricidal properties, both by the variable exposure method and the kymograph method.

One of these, 2-ethyl-4-chloro-6-hexylresorcinol, seems to be more active than hexylresorcinol *in vitro*.

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ARTIFACTS IN TRANSPARENT ADHESIVE TAPES USED FOR PERIANAL PINWORM SWABS¹

DOROTHY J. HITCHCOCK

The use of transparent adhesive tapes for swabbing the perianal skin in the diagnosis of pinworms, as described by Graham (1), has brought about a microscopical study of these tapes for artifacts simulating *Enterobius vermicularis*, pinworm eggs.

The artifacts simulating pinworm eggs found in "cellophane" (non-adhesive) used in the N. I. H. swab were illustrated by Reardon (2).

A microscopical examination was made of two brands of $\frac{3}{4}$ inch transparent adhesive tape, "Scotch Cellulose" tape² and "Texcel Cellophane" tape.³

In the preparation of the unused tape for microscopical examination precautions were taken not to introduce artifacts on the slides from the handling of the preparation or dust from the air.

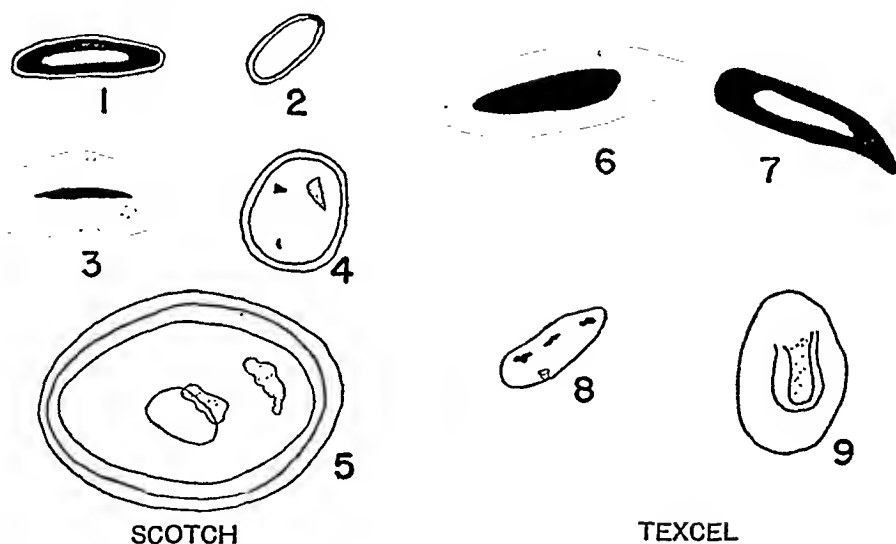


FIG. 1. UNUSED TAPES

Two, $\frac{3}{4} \times 5$ inch, pieces of tape were cut from six rolls of each brand of tape. These pieces were placed on new chemically cleaned, 1×3 inch microscope slides, stretched and pressed with lens paper in order to prevent finger prints and the minimum of air bubbles. The center $2\frac{1}{2}$ inches of the tape was examined systematically under the microscope, $100\times$, for artifacts (Fig. 1). Thirty inches of each brand of unused tape, total area 22.5 square inches, were thus examined.

Eight slides, prepared with each brand of tape, were examined for artifacts

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² Minnesota Mining and Manufacturing Company, St. Paul, Minnesota.

³ Industrial Tape Corporation, New Brunswick, New Jersey.

(Fig. 2) after being used for swabbing the perianal region for the recovery of pinworm eggs. These swabs were taken by the mothers and precautions against the introduction of extraneous matter could not be so rigidly followed as in preparing the unused tape preparation in the laboratory.

On the unused and used tape preparations the most commonly encountered artifacts were bubbles (Figs. 1 and 2), Nos. 1, 3, 6, 7, 13, 15 and 16 having a size and shape similar to pinworm eggs. The bubble wall is greenish-black, wide, and has a depth of focus greater than an egg.

The artifacts imbedded in the unused and used tapes (figs. 1 and 2), Nos. 2, 4, 8, 9, 10, 11, 12 and 14 somewhat resemble *Enterobius vermicularis* eggs in size, shape and the thin, transparent wall, but do not contain larvae. The artifact No. 5 does not resemble a pinworm egg but was of interest because it was found only on used and unused "Scotch Cellulose" tape.

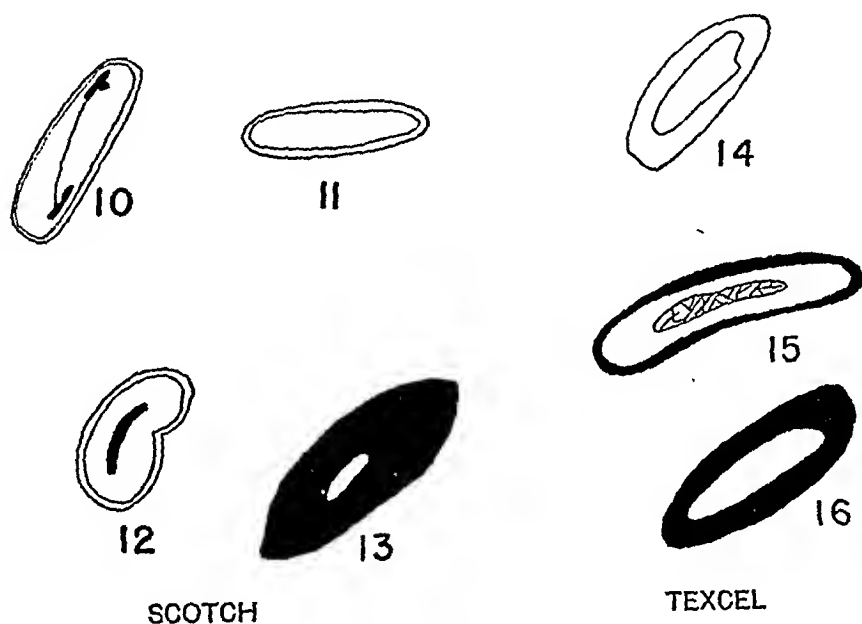


FIG. 2. USED TAPES

No differences were found in the number of artifacts resembling pinworm eggs in the two tapes. The few artifacts found can be easily differentiated from *Enterobius vermicularis* eggs after a careful study of the eggs on transparent adhesive tape preparations.

SUMMARY

A microscopical study was made of two brands of transparent adhesive tapes for artifacts resembling *Enterobius vermicularis* eggs. Very few artifacts, other than bubbles, simulating pinworm eggs were found.

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POISONING BY SCORPION STINGS IN ISRAEL

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The present article is based on observations made on 22 cases of poisoning due to scorpion stings during the years 1935-47. Seventeen of the cases were seen by the author himself, (7 of them were published in local medical journals in 1936 (3), and 1937 (4)), whilst descriptions of the remaining 5 cases were supplied by various practitioners from Jerusalem and Tiberias. Nine of the cases were treated at the Hadassah Rothschild University Hospital in Jerusalem, where the author previously worked, and 10 at the P. Schweitzer Hospital in Tiberias. The remainder were not hospitalized.

A search of the medical literature has not disclosed any clinical description of scorpion stings in Israel, with the exception of a brief report by J. Gurevitz in 1924 (6).

Many cases of scorpion stings occur in this country every year, but in the following we shall deal only with those that were accompanied by manifestations of generalized intoxication. The relative incidence of the latter is not known, since cases in which scorpion stings cause only local reactions are not recorded. However, in order to give a rough idea, the author would like to mention that every year he has encountered scores of cases of stinging by scorpions, and yet, in the course of twelve years, he has observed only 22 cases of generalized poisoning.

According to Shulov (13), 12 species of scorpions have been identified in Palestine, of which the most frequently occurring are: 1) *Buthus quinquestriatus*, 2) *Buthus judaicus*, 3) *Prionurus bicolor*, 4) *Scorpio maurus* and 5) *Nebo hierichonticus*.

Shulov found (13) that the sequence of the various species according to their toxicity in white mice was as follows: 1) *Buthus quinquestriatus*, 2) *Prionurus bicolor*, 3) *Nebo hierichonticus*, 4) *Buthus judaicus* and 5) *Scorpio maurus*.

In the cases under review, 11 of the scorpions were identified, ten being *B. quinquestriatus* and one *Prionurus crassicauda*. An additional scorpion was seen but not identified; the relatives of the patient stated that it had been a large, black specimen. It is worth noting that all the severe cases and fatalities were caused by the stings of *B. quinquestriatus*.

A classification of the cases according to seasons of the year reveals that all of them occurred during the months June to December, (June, 1; July, 3; August, 3; September, 7; October, 6; November, 1; and December, 1). The largest number of cases thus occurred during the hot season.

The age of the affected persons was from 6 months to 63 years; up to 1 year, 1; from 1 to 4 years, 6; from 4 to 6 years, 4; from 6 to 9 years, 4; 9 years and upwards, 7 (including a youth of 15, a woman of 30 and an old man of 63). Seventeen of the patients were male and 5 female.

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Table I shows a summary of clinical manifestations in the order of their frequency. In Table II the manifestations have been classified according to their

TABLE I

Summary of clinical manifestations

Figures in parentheses indicate the number of cases in which the symptom was observed.

Hyperirritability.....	(19)	Shivering.....	(4)
Vomiting.....	(17)	Areflexia.....	(3)
Polypnoea.....	(16)	Hypertension.....	(3)
Hypersalivation.....	(14)	Ptosis of the lid.....	(2)
Profuse perspiration.....	(13)	Shock.....	(2)
Local pains.....	(9)	Flatulence.....	(2)
Cyanosis.....	(8)	Pallor.....	(2)
Myoclonic twitchings.....	(8)	Generalised erythema.....	(2)
Flushing of the face.....	(7)	Local hypaesthesia.....	(2)
Hyperthermia.....	(7)	Abdominal tension.....	(2)
Priapism.....	(7)	Hypothermia.....	(2)
Thirst.....	(6)	Rhinorrhoea.....	(2)
Clouding of consciousness.....	(5)	Miosis.....	(2)
Tachycardia.....	(5)	Pupillary areflexia.....	(1)
Retentio urinae.....	(5)	Involuntary defaecation.....	(1)
Mydriasis.....	(5)	Pollakiuria.....	(1)
Meteorism.....	(4)	Somnolence.....	(1)
Spastic speech.....	(4)	Sleeplessness.....	(1)
Bradycardia.....	(4)	Piloerection.....	(1)
Hyperreflexia.....	(4)	Paraesthesia.....	(1)
Abdominal pains.....	(4)	Nystagmus.....	(1)
Frothing at the mouth.....	(4)	Swelling of the lip.....	(1)
Cold extremities.....	(4)	Exophthalmus.....	(1)

TABLE II

Summary of clinical manifestations subdivided according to physiological systems

Respiration: Polypnoea.

Circulation: Cyanosis, vaso-constriction, vaso-dilatation, bradycardia, tachycardia, hypertension, shock.

Digestion: Hypersalivation, thirst, vomiting, abdominal pains, abdominal tension, meteorism, flatulence, involuntary defaecation.

Uro-genital system: Priapism, urinary retention, pollakiuria.

Thermo-regulation: Hyperthermia, hypothermia, shivering, piloerection, cold extremities, profuse perspiration.

Disturbances of Consciousness: Clouding of consciousness, hyper-irritability, sleeplessness, somnolence.

Motoric Disturbances: Myoclonic twitchings, spastic speech.

Disturbed Reflexes: Hyper-reflexia, a-reflexia (including pupillary a-reflexia).

Sensoric Disturbances: Local pains, hyperaesthesia, paraesthesia.

Ocular Disturbances: Miosis, mydriasis, nystagmus, ptosis, exophthalmus.

Skin: Swelling of the lip, local and generalized erythema.

physiological relationship. Table III contains the results of the few laboratory examinations that were carried out.

TABLE III
Results of laboratory examinations

A) Blood (examined in 4 cases)				
	Haemoglobin gm.	Erythrocytes mill.	Leucocytes	
1	13	4.4	15,000	
2	12	3.7	42,000	
3	12	4.7	70,000 (lymphocytosis)	
4	15	4.58	16,800 (eosinophilia)	
B) Urine (examined in 8 cases)				
	Albumen	Sugar	Urobilinogen	Sediment
Positive.....	1	—	2	In two
Negative.....	7	8	6	cases a few leucocytes.
C) Spinal fluid (a single case examined)				
Pressure: Increased.				
Cells: Normal number.				
Pandy				
Nonne-Apelt				
Weichbrodt				
Tests: Negative.				

In the following, a few typical clinical histories will be described in detail.

Case 1. T. A., an Arab infant, 6 months old. Admitted to the P. Schweitzer Mem. Hospital on 28/9/1945. About 20 minutes before being admitted to hospital, he had been stung by a big, yellow scorpion. The accident occurred when his mother was drying him after his bath. The baby suddenly began to cry, and his mother unrolled the towel to see what had happened. At this moment, the mother, too, was stung in the hand, and a scorpion, found in the towel, was killed and identified as a large specimen of *B. quinquestratus*.

10:50 a.m. Admitted to the hospital—a well-developed infant in a good state of nutrition. Marked restlessness. The infant throws himself from side to side. Saliva flows from his mouth. Respiration is labored, the auxiliary respiratory muscles taking an active part in it. The supra-sternal notch is drawn inwards during respiration. Lips and finger nails are cyanosed. The skin of the extremities is reddened and resembles gooseflesh. Tension of the abdominal wall is normal.

11 a.m. Same as above. Around the mouth, in the small facial muscles, a delicate fasciculation makes its appearance.

11:35 a.m. Injection of $\frac{1}{2}$ ampoule of Gynergen and $\frac{1}{2}$ mg. of atropin. From time to time the infant has fits of spastic coughing. Insufflation of oxygen.

12:00 noon. Priapism, restlessness more pronounced. Intermittent cyanosis, together with fits of spastic coughing. Fasciculation around the mouth is maintained.

12:15. Subcutaneous injection of $\frac{1}{2}$ ampoule of Diallyl 'Teva', (Diallyl Barbituric Acid 0.2).

13 p.m. Restlessness continues. Vomiting. A further $\frac{1}{2}$ ampoule of Diallyl injected.

13:35. Persisting restlessness, vomits dark-brown material. Temperature reaches 42°C. Pulse no longer palpable.

13:40. Exitus. Respiration ceased first, then cardiac action. Postmortem examination was not permitted.

Summary. Generalized intoxication due to a scorpion sting in a 6 months-old infant. Symptoms of poisoning appeared some 20 minutes after the stinging. The most characteristic signs were: restlessness, labored respiration, cyanosis, priapism, vomiting, fasciculation of the facial muscles, spastic coughing and a pre-terminal rise of temperature to 42°C. Died three hours after having been stung. The treatment adopted in no way influenced the course of the intoxication (anti-toxic serum was not available).

Case 2. H. Z., A Jewish boy, 6 years old. Admitted to the P. Schweitzer Mem. Hospital on 15/10/1944.

13:30 p.m. An hour previously he had been stung in a finger of his right hand by an unidentified scorpion. On admission, he was found to be of pasty complexion and in a fair state of nutrition. His face was flushed, and rhinorrhoea and vomiting soon commenced. The boy was highly excited, and could be confined to bed only with difficulty, throwing himself continually from side to side.

13:50 p.m. Subcutaneous injection of $\frac{1}{2}$ ampoule of Gynergen and $\frac{1}{2}$ mg. of atropin.

14 p.m. Shivers with cold. (The room temperature was far from cold.) Sweats profusely. Short myo-clonic twitchings of the labial muscles. Light polynoea, abdomen soft. Auscultation and percussion of the lungs reveal no abnormalities. Rhinorrhoea continues, a watery, limpid discharge flowing from the nose. Tendon reflexes greatly exaggerated. Babinski's reflex absent.

14:45 p.m. No change in the general condition. Rhinorrhoea continues. Face still flushed. Complaints of feeling cold. A further injection of $\frac{1}{2}$ ampoule of Gynergen and $\frac{1}{2}$ mg. of atropin was given.

15 p.m. Calmed down and fell asleep. Nasal discharge diminished.

16 p.m. General feeling good. Resting.

On 16/10/44 the boy left hospital, fully recovered. The tendon reflexes were no longer exaggerated.

Summary. Poisoning due to a scorpion sting in a 6 year-old boy. *Main symptoms:* State of excitement, flushing of the face, rhinorrhoea, feeling of coldness, vomiting, rapid breathing. The symptoms appeared an hour after infliction of the sting, and persisted for about three hours.

Case 3. Sh. L., A Jewish boy, aged 11. Admitted to the P. Schweitzer Mem. Hospital on 30/8/41 at 21:40. A quarter of an hour previously he had been stung by an unidentified scorpion in the big toe of his left leg. He at once felt very severe pains at the site of the sting, and after 10 minutes began to vomit, his face became flushed, he became excited and his breathing laboured. Cold perspiration covered his body and he complained of feeling chilly. In this condition he was admitted to hospital. His state of nutrition was good. His face was very flushed, except for a narrow area around his mouth. His lips were slightly cyanosed. He vomited incessantly and was in a state of great excitement. His pupils were dilated, but reacted to light. Auscultation and percussion of the lungs revealed nothing abnormal. The heart sounds were clear and cardiac action was regular—72 beats per minute. The tendon reflexes were exaggerated. The Babinski reflex was negative. Nothing could be seen at the site of the sting.

22:00. Face still flushed; in a state of extreme excitement. Vomits profusely a viscid material. Subcutaneous injection of one ampoule of Gynergen and $\frac{1}{2}$ mg. of atropin.

22:30. Vomiting continues; increasing restlessness. The patient now feels hot, and the pulse rate has risen to 120 per minute. The pulse becomes irregular. Subcutaneous injection of 0.2 cc. of Sevalnal.

23:00. Still vomiting, mostly bile. Feels chilly again. Reddish spots appear on various parts of the skin. Still very excited. Voice hoarse.

23:30. Intravenous drip infusion of saline is commenced, also intravenous injection of one ampoule (5 cc.) of anti-toxic serum (prepared by the Pasteur Institute of Algiers).

24:00. Condition improved. Vomits more infrequently. Very thirsty.

31/8/41. General condition—good. Pulse—normal. Saline infusion discontinued. Temperature 37.7°C.

1/9/41. Left hospital, fully recovered.

Summary. Generalized intoxication due to a scorpion sting in an 11 year old boy. The first symptoms appeared 10 minutes after the sting and persisted for 2½ hours. The most striking symptoms were: Excitement, flushing of the face, profuse vomiting, sweating, feeling of chilliness and exaggerated tendon reflexes. The patient was treated with anti-toxic

serum, and the impression was gained that the change for the better followed closely upon the administration of the serum.

Case 4. A. J. A Persian woman, aged 30. Admitted to the P. Schweitzer Mem. Hospital on 31/10/40, at 20:00. She had been stung by a large, yellow scorpion which was killed and identified as a specimen of *Prionurus crassicauda*. The site of the sting was close to the big toe of the left foot. The woman felt very severe pains at once. A short time after the infliction of the sting she began to vomit and complained of abdominal pains. She vomited all night and felt formication in her fingers and toes. Breathing was laboured and speech difficult. Towards the morning the patient entered a state of general excitement, which was relieved by a subcutaneous injection of 0.01 gm. of morphine. At noon her condition deteriorated again and her pulse became accelerated (160/minute). Respiration was also hurried.

Following the subcutaneous injection of 1 ampoule of Gynergen and $\frac{1}{2}$ mg. of atropin, the patient felt easier. The pulse rate returned to 90/minute, and she fell asleep. In the afternoon, the feeling of formication returned and the patient entered into a state of total exhaustion, where even eating was an effort. The temperature was 38°C.

The state of general weakness continued for another 2-3 days, by which time other signs of intoxication had disappeared.

Summary. A 30 year old woman, weighing 79 kg., was stung by a scorpion of the *Prionurus crassicauda* species. A short time after the sting, the following symptoms appeared: Abdominal pains, vomiting, extreme excitement, accelerated pulse, paraesthesia. The symptoms of poisoning persisted for almost 24 hours, and when they had passed, weakness was felt for another few days.

TREATMENT

In the first few years of the period under review no specific anti-toxic serum was at our disposal, and, consequently, symptomatic treatment had to be resorted to. In 5 cases we used serum anti-venimeux, prepared against North-African snakes. Although all these cases recovered, we were unable to observe any marked beneficial results ascribable to the use of the serum. In 5 other cases we tried the effect of Atropin and Gynergen injections, on the basis of animal experiments carried out by Ahmed Hassan Mohammed and Ali Hassan in 1940 at Cairo (7). These workers devised their experiments on the assumption that scorpion toxin acted as strong stimulant of the autonomic nervous system. Rats and dogs which had received lethal doses of the toxin survived when given injections of atropin and ergotoxin within 20-90 minutes after administration of the poison. The authors propose the adoption of this line of treatment for human beings.

As already mentioned, we tried this line of treatment in 5 cases. In three of them we failed to notice any change in the condition of the patients, whilst in the remaining two we gained the impression that an improvement was noticeable after the injection of atropin and Gynergen. The injections were administered soon after the admission of the stung persons to hospital, and were repeated in an hour's time, if necessary.

In a further 5 cases we used an anti-toxic serum prepared against scorpions of North Africa (Pasteur Institute of Algiers). Of these 5 cases, one died, whereas of 17 other cases where serum was not used, five died. Although the quantity of serum administered was rather small (1-2 ampoules of 5-10 cc.),

we were able to observe in every instance a marked improvement a short time after administration. In the case that terminated fatally, the quantity of the serum was, of course, insufficient. It should be noted that the serum was prepared against the toxin of North African scorpions, and yet it proved of value. Shulov (14) found that serum prepared by the injection of toxin of a local specimen of *Buthus quinquestratus* into rabbits was three times more active than serum prepared in Egypt against the same species of scorpion.

The manifold experiments of Etienne Sergent of the Pasteur Institute of Algiers, have established the value of sero-therapy, and it devolves upon the authorities in this country to prepare specific anti-sera against the local scorpions in sufficient quantities.

POISONING BY SCORPION STINGS IN OTHER COUNTRIES

I have not had access to all that has been published on this subject, and I propose to confine myself to a short review of the literature of recent years. Kent and Stahnke (8) (1939), of the State of Arizona, U. S. A., report on 75 cases of scorpion stings admitted to the Southside Hospital during the years 1930-1937. The most poisonous scorpion in their experience was *Centruroides sculpturatus*, the sting of which was almost always fatal to children, if they were less than 1 year old, unless proper treatment was applied. The symptoms described are as follows: Severe pains at the site of the sting, with no apparent local changes, restlessness, tension of the abdominal muscles, tonic twitchings of hands and legs, hyper-salivation, accelerated pulse, pyrexia, dyspnoea, cyanosis, involuntary urination and defaecation. In cases that recovered, symptoms disappeared in the course of 12 hours.

Symptoms in adults were less serious. At times, some hyperaesthesia remained at the site of the sting. The authors applied mainly symptomatic treatment (atropin, barbiturates, compresses using a concentrated solution of ammonium hydroxide, etc.). Only more lately did they resort to anti-toxic serum, with encouraging results.

Gungle, also of Arizona, describes scorpion poisoning in a personal communication, as follows (5) (1939): In his experience, the most important scorpion was *Centruroides gracilis*. Stings were more dangerous in the summer, and especially in young and weak individuals. Symptoms: tonic/colonic twitchings, hyper-salivation, a feeling of choking and serous meningitis, with increased pressure in the cerebro-spinal fluid. By way of treatment, he suggests lumbar puncture for the relief of cerebro-spinal pressure, and barbiturates. All patients who received this treatment recovered.

O. de Magalhães, of Belo Horizonte, Brazil (1938-9) (9) mentions statistical data on 6,668 cases in Brazil, of which 327 were fatal. The age extremes were 60 days and 104 years, both cases concerned terminating fatally. The important scorpions in Brazil belong mostly to the genus *Tityus* (C. L. Koch), viz. *serrulatus*, *bahiensis*, *dorsomaculatus*, etc. The toxin gives rise to disturbances of the nervous system and other organs: general exhaustion, state of excitement, dizziness, vomiting, hypersalivation, pupillary disturbances, nystagmus, choking, dis-

turbances in speech, severe pains at the site of the sting, hyperaesthesia, circulatory and respiratory disturbances, hiccoughs, profuse sweating, nasal and conjunctival discharge, increased bronchial secretion, rise in blood sugar, glycosuria, generalised convulsions, involuntary urination and defaecation, hypothermia, or rises in temperature over 38°C. The customary line of treatment was serotherapy.

Caius and Mhaskar (1932) (3) report their views on the scorpions of India. According to these authors, the important scorpions in that country belong to the genus *Buthus*, (*B. europaeus*, *B. quinquestriatus*), or to genus *Palamneus*. It is only rarely that scorpion stings cause death, and then only in children. The effect of the scorpion toxin is reflected in a state of hypersensitivity of the nervous system: severe pain, exaggerated reflexes, shivering, tremor, convulsions and finally paralysis. Other phenomena are: excessive lachrymal, nasal and salivary secretion, involuntary urination and defaecation, vomiting, coughing diarrhoea, and disturbances in breathing, terminating in respiratory paralysis. Contraction of the blood vessels leads to increased blood pressure.

U. P. Basu of Calcutta (1939) (1) describes 19 cases of stinging by scorpions which were admitted to hospital during the years 1928-37. According to him, the poisonous scorpions belong to the family Buthidae. The toxin has the following effects: Severe pain at the site of the sting, head ache, giddiness, nausea, vomiting, profuse perspiration, a feeling of chilliness, cold extremities, hypothermia, weak pulse. At times there is loss of consciousness. In serious cases respiration is hurried. Altogether, five of the patients died, all of them children, and in the majority of these pulmonary oedema supervened. General, symptomatic treatment was applied.

J. A. Waterman of Trinidad (1938) (15) reports that, during the years 1929-33, 658 cases of scorpion stings, 33 of them fatal, were recorded in one of the hospitals. Mortality thus amounted to 4.7 per cent. The most poisonous scorpion in Trinidad is the "black" one (scientific definition?). Mortality was highest in children, and death occurred from half an hour to 42 hours after infliction of the sting. Symptoms of poisoning were: Severe pains at the site of the sting, hypersalivation, nausea, vomiting, bradycardia, subnormal temperature (but sometimes pyrexia), hurried breathing. Cyanosis was not observed. Sweating was profuse and sometimes excitement was pronounced. Often abdominal pains and tenderness were observed, and the abdominal wall was tensed. Singultus was recorded a number of times, and convulsions were fairly frequent in children, especially in severe cases. At times monoplegia and hemiplegia were encountered, and transient blindness was also met with. Hyperglycaemia and glycosuria are probably due to changes in the pancreas, where edema or inflammation are observed. The author states that the scorpion toxin is excreted in the milk of the nursing mother and is likely to endanger the life of the infant. The cause of death is respiratory or circulatory failure. The author suggests local treatment—intermittent application of the tourniquet for short periods, potassium permanganate, and general measures (saline or glucose solution administered parenterally, sedatives, etc.).

Etienne Sergeant, of Algiers (1939-46) (10-11) has collected observations on thousands of cases of scorpion poisoning from various physicians throughout Algiers, and publishes them every year in the Arch. de l'Institut Pasteur d'Algerie. According to him the most important scorpions in his country from the medical point of view are: 1) *Prionurus australis*, Linne; 2) *Prionurus amoreuxi*; 3) *Prionurus liouvillei*, and 4) *Buthus occitanus*. The author has performed numerous experiments with scorpions and has contrived to persuade physicians about the necessity of using anti-toxic serum. He has recorded interesting observations on the symptoms of scorpion poisoning: severe pain at the site of the sting, dizziness, profuse sweating combined with a feeling of chilliness, pallor followed by cyanosis, severe headache, and sometimes myo-clonic twitchings in the extremities. Temperature may remain normal, even in the most severe cases, but hypothermia or hyperthermia may also be seen. Other symptoms are: respiratory disturbances (polypnoea), acute pulmonary oedema, tachycardia, nausea, vomiting, severe abdominal pains, excitement or coma, meningismus, and pupillary disturbances. In his many articles published throughout the years, the author has proved the effectiveness of the anti-toxic serum.

The above review of literature goes to show that despite the fact that the articles concerned were published in different countries and even in different continents, and that the scorpions mentioned belong to diverse species, the manifestations of poisoning exhibit a striking resemblance.

SUMMARY

1. The clinical manifestations in 22 cases of poisoning by scorpions are summarized. Some of these occurred in the southern part of Israel (Jerusalem and vicinity) and the others in the northern part (Tiberias and vicinity). These cases were observed by the author in the course of 12 years (1935-47).

2. A study of the symptoms indicates that the majority of them originate in the vegetative nervous system.

3. Of the 22 cases, six (aged 6 months to 12 years) terminated fatally.

4. All the severe cases, including the fatalities, were caused by *Buthus quinquestratus*.

5. A detailed description of four cases is presented.

6. The clinical literature on scorpion poisoning is reviewed.

7. Treatment applied was largely symptomatic. In five cases, a combination of atropin and Gynergen was administered, based on the work of Ahmed Hassan Mohammed and Ali Hassan (Cairo) (1940). In three of the cases, this line of treatment failed to produce any effect, whilst in the two remaining instances an improvement was noted after administration. In 5 cases, anti-toxic serum, produced by the Pasteur Institute of Algiers, was injected. Of these cases, one died, as compared with 5 fatalities out of 17 cases that did not receive specific treatment.

The quantity of serum given was insufficient.

8. The author stresses the need for the production of an anti-toxic serum specific for the scorpions of his country.

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PLANORBIDAE RECORDS OF THE NETHERLANDS ANTILLES

EDWIN VAN DER KUYP

The Territory of Curaçao or the Netherlands Antilles consist of two groups of islands:

1. The Netherlands Leeward Islands: Curaçao, Aruba and Bonaire, which are situated near the northern coast of Venezuela.

2. The Netherlands Windward Islands: the southern part of St. Martin (the northern part is French), Saba and St. Eustatius, which are situated between the American Virgin Islands and Guadeloupe.

The total annual rainfall in Curaçao is scanty, the average for the 18 year period, 1930-1947 being 570.6 mm. But in all the plantations groundwater is pumped from wells with the aid of windmills and stored in concrete reservoirs. From 1941 to 1947 *Tropicorbis isthmicus* Pilsbry (1) was found to be very widely distributed in most of the plantations on the island of Curaçao. *Tropicorbis isthmicus* was found in many of these reservoirs, in pools near the reservoirs and windmills, in springs, ditches, ponds, wells, in concrete tanks with water for stock animals, and even in barrels. The pH of the water varied from 6.5-9.6, and the chlorine content from 50-2,600 mgrm. per liter. On the island of Bonaire, *Tropicorbis isthmicus* was found in Pos di Ichi, a well near the capital, Kralendijk, in April 1943. It may have been *Tropicorbis isthmicus* Pilsbry that was previously reported under the names *Planorbis pallidus* and *P. circumlineatus*. But probably there are other species yet to be discovered. The following authors published records on Planorbidae of the Netherlands Leeward Islands: Smith (2), of a *Planorbis* collected by Ernst Hartert in Curaçao in 1892, while Vernhout (3), mentioned Smith's work; Baker (4), *Planorbis pallidus* Adams (syn. *P. pallidus* Chessin, *P. circumlineatus* "Shuttleworth" Chessin), in Curaçao and Bonaire; Van Benthem Jutting (5), *Planorbis circumlineatus* Shuttleworth (syn. *P. pallidus* Baker), collected by C. J. van der Horst in Curaçao in 1920; Jutting stated: "*Planorbis* seems even to abound in this island"; Baker (6), *Planorbis pallidus* in Curaçao; Wagenaar Hummelinck (7), *Planorbis circumlineatus* Shuttleworth, collected in Curaçao and Bonaire in 1930; Richards and Wagenaar Hummelinck (8), *Planorbis circumlineatus* Shuttleworth, (syn. *P. pallida* Adams), in Curaçao and Bonaire; and Wagenaar Hummelinck (9), *Planorbis circumlineatus* Shuttleworth, found in many plantations in Curaçao in 1936, and in Bonaire in 1936 and 1937.

The genus *Tropicorbis* is not considered of primary importance in the transmission of *Schistosoma mansoni*. Morrison (10) stated that *Tropicorbis isthmicus* Pilsbry is not an effective intermediate host of this parasite in Panama. Attempts by the author to infect this snail with *Schistosoma mansoni* from an imported case were unsuccessful.

Australorbis glabratus lugubris Wagner, and *Helisoma duryi intercalare* Pilsbry, were imported to Curaçao from Venezuela by a school teacher in 1941. The first species is known as a dangerous intermediate host of *Schistosoma mansoni*,

but the *Helisoma* species is not considered as such. Cram, Jones and Wright (11) could not infect this species. The teacher distributed these two species among schoolboys to be reared in their aquaria. These aquaria, usually glass containers, such as empty battery jars, but also concrete tanks in the ground, were a very popular hobby of not only children, but also of adults at that time. From the aquaria they were introduced deliberately or accidentally into the concrete reservoirs of several plantations. They were not found infected with *Schistosoma mansoni*. The same *Australorbis* species was found only once in Aruba in an aquarium at St. Nicolaas.

Marisa cornuarietis Linnaeus was found at St. Patrick in Curaçao. Although it is *Planorbis* shaped it is not a *Planorbis*.

Autochthonous cases of schistosomiasis have never been reported from the Netherlands Leeward Islands (12, 13, 14, 15), but there is a potential danger in that many immigrants from known endemic areas are present in Curaçao and Aruba, and sewage disposal methods are very primitive in rural parts of these islands.

In 1890 Mazé (16) made a survey of the snails in St. Martin, but did not mention any *Planorbis*. Between 1923 and 1927 Emanuels (17) found *Planorbis olivaceus* (?) at Cul de Sac in Dutch St. Martin, and at Colombier in the French part of the island. He also reported several cases of schistosomiasis *mansoni* from Colombier. He collected snails from wells and ponds in this village which were found to be infected with *Schistosoma mansoni*. However, no indigenous case has ever been reported from Dutch St. Martin, nor from the other Netherlands Windward Islands (12, 13, 14). In 1930 Hoffman (18) reported schistosomiasis from Colombier in French St. Martin. This information he got from the French Commissioner. Faust (15) mentioned Hoffman's report.

SUMMARY

Tropicorbis isthmicus Pilsbry is found on the islands of Curaçao and Bonaire. Other *Tropicorbis* species may also be present. *Australorbis glabratus lugubris* Wagner, and *Helisoma duryi intercalare* Pilsbry were imported into Curaçao from Venezuela. The *Australorbis* species was found only once in Aruba in an aquarium. Autochthonous cases of schistosomiasis have never been reported from the Netherlands Antilles.

ACKNOWLEDGMENTS

The author is indebted to the following members of the staff of the Department of Mollusks of the Academy of Natural Sciences of Philadelphia: Dr. Henry A. Pilsbry, who identified the *Tropicorbis* species, Dr. H. B. Baker, who identified the *Australorbis* species, and to Mr. Ch. B. Wurtz and Mr. R. A. McLean for their assistance. The author is also indebted to Dr. J. P. E. Morrison of the U. S. National Museum in Washington for his assistance in identifying the *Helisoma* and *Marisa* species.

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AN APPEAL TO OUR READERS

Just as the society convened in the annual meeting in New Orleans last December, the Department of Parasitology of the University of Santiago, met with disaster. The department is headed by Dr. Amador Neghme, well known to many of our readers. The following paragraph was prepared by Dr. E. C. Faust, from information supplied by Dr. Neghme. It is hoped that many members of the society and others may be able to assist Dr. Neghme in re-establishing their teaching collections and library.

"On December 5, 1948, early in the morning, fire destroyed the School of Medicine, The Biological Institute and The Department of Parasitology of the University of Santiago, Chile. The Department of Parasitology lost their research microscopes, glassware and instruments including new equipment which had just arrived from France; also more than 5000 microscopic preparations of parasite material and the museum and teaching collection of bottle specimens. The Parasitological Library, including all volumes and reprints, was a total loss, as were several manuscripts in preparation and part of the registers of surveys on Chagas' disease, comprising 15,000 people, of whom 1761 were positive for the infection. Dr. Amador Neghme, Professor of Parasitology, appeals to all workers in the field to send him any specimens and microscopic slides, likewise any available reprints and Journals. Please send the material, properly packaged and properly labelled to:

Professor Amador Neghme
P. O. Box 9183
Santiago, Chile"

It is suggested that those inclined to assist, communicate with the Cultural Attaché of the Chilean Embassy in Washington, D. C., to ascertain whether such material could be forwarded by the embassy from Washington to Chile through official channels.

BOOK REVIEWS

The Leptospiroses. By P. H. VAN THIEL, pp. x plus 202 plus 21 bibliography plus 7 index with 19 figures and 5 textfigures. University of Leiden, Netherlands, 1948. (Price, f.16.50.)

This authoritative monograph by the Professor of Parasitology of the University and the Institute of Tropical Medicine at Leiden is a valuable contribution to a field which has heretofore received only fragmentary attention. The many clinical types of the disease produced by leptospirae, the epidemiology of these infections and the biological and immunological relationships of the many species or strains of leptospirae are brought together in a very comprehensive manner.

After introducing the subject by considering the morphology, classification and technique of diagnosis, a long chapter is devoted to the general epidemiology of the infections including the role of carriers, infection of man by direct infection and by contact with infected water, the mechanism of infection, the isolation of leptospirae from water and the significance of avirulent but potentially pathogenic strains living in water. Then follow chapters on prophylaxis, pathogenesis, general clinical aspects and therapy of these infections. Following this are chapters describing the individual diseases caused by recognized species or varieties of leptospirae in various parts of the world, including the much debated disease in which icterus and hemoglobinuria occur. Finally there is a chapter on leptospiroses caused by strains found only in animals and perhaps occasionally occurring in man. Tables at the end of the book show the geographical distribution of the leptospiroses and the relation to individual animal reservoirs. This book should be in every medical library and will be very useful to research workers and diagnostic laboratories. Its only drawback is that the author himself has prepared the English text, with the result that his statements are frequently not made in idiomatic English. This, however, is greatly counterbalanced by the authoritative and scholarly handling of the subject.

HENRY E. MELENEY

The Sick African. A Clinical Study. By MICHAEL GELFAND. 2nd revised edition. 124 figs. 699 pp., 1948. Cape Town, S. Af. Stewart Printing Co. Ltd. 38 shillings.

While directed primarily to the physician undertaking practice in Africa for the first time, many general remarks about the African patient will strike a familiar chord with some doctors in the southern states. A second, much enlarged, edition of a book published in 1943 indicates a favorable reception and fundamental usefulness of the first edition. The illustrations are frequently excellent. A number of typographical errors and mis-spellings can be noted. It is not clear why native is spelled with a (caps) "N" throughout. The binding is unsuitable for very moist climates, becoming soggy when humidity is high.

The arrangement of the material endeavors to follow clinical systems rather than classification of the infective agents. Thus, a certain amount of repetition occurs which is unavoidable and some diseases appear among unusual companions. It should be noted that it is written from the viewpoint of a clinician who is also a pathologist with considerable autopsy experience. An especially apt statement appears in the section under malignant disease in the native, viz.: "As facilities for a proper pathological report are denied to the greater part of Africa, it is clear that it will take a considerable time before the figures of malignant disease in the native will be sufficiently numerous for definite conclusions to be drawn".

The description of the patient and his attitude as well as the exposition of disease make this book valuable for the medical newcomer to Africa.

The following comments seemed worthy of mention.

Relative incidences of disease are vaguely stated as "common", "uncommon", etc.

In malaria, after describing the differences between immune and non-immune peoples

brief directions for diagnosis are given. The combined thick film-thin smears are still recommended despite the fact no single stain does justice to both. It is doubtful Giemsa solution made up daily will be satisfactory three hours after mixing. A somewhat enigmatic statement occurs later. "Often the recognition of the particular parasite, clinically, is not easy and usually impossible without examination of blood smears." Under distribution of disease, quartan malaria is cited as relatively uncommon. Actually it is widely found but the number of cases is small and falciparum is correctly stated as the only parasite of importance in Africa. Treatment still begins with quinine, atabrine and plasmochin. Chloroquine is not mentioned and paludrine is mentioned as inferior although the dosage stated seems inadequate. That atabrine *in adequate dosage* acts more quickly on vivax and quartan than falciparum is misleading. So is the inference that only quinine intravenously is indicated in cerebral malaria. The rationale of *plasmochin* usage is never explained.

"Much more frequent than congenital malaria is infection of the placenta by the parasite. It is possible that an infant born when the placenta is infected may be immunized to malaria by it." This statement reflects the belief of Wright in Sierra Leone who observed the series of Blacklock and Gordon which included 8 sets of twins. In Africa, the second twin has a poor chance of survival.

The types of blackwater fever are the authors own.

No mention is made of flotation techniques in stool examinations. Centrifugalization is the only concentration method described. The distribution of nuclei around the center of the cyst as the feature of differentiation of *histolytica* will not find many adherents. Sig-moidoscopy is given appropriate attention. The book rightly stresses the frequency of amebic liver abscess in the native. The frequent mention of carbontetrachloride in the treatment of hookworm suggests a preference for this drug over tetrachlorethylene which is stated to be less toxic. It is doubtful if the continued use of santonin in the treatment of ascaris and pinworm infections can be recommended. Although giardia is treated with atabrine, strongyloides is stated to be harmless.

It might be mentioned that yaws occurs also where the humidity is high as well as the temperature. No reference to the toxicity of the diamidines was noted, nor mention, of the use of PABA in the treatment of rickettsial diseases. Schistosomiasis largely reflects the author's own experience and is capably described.

Nutritional disorders and the part played in other diseases are well brought out. Little stress is placed on the fact that although sub-optimal, the diet of the rural native is often far superior to that of his kinsman who moves to urban areas. There high prices prevent him getting an equivalent diet in spite of the fact that wages may be many times higher than in his home community.

Onyala, is evidently a disease of some frequency in Southeast Africa. The chapter on tuberculosis, the second most important disease in Africa, contains considerable theoretical discussion of questionable value. Tuberculosis presents no new features in Africa but varying customs, diet, conditions and changes do influence it in strange ways. It would seem that this experience is chiefly of South Africa and his descriptions of findings in other parts of Africa are largely obtained from the literature.

ARTHUR J. WALKER

Malaria Control on Impounded Waters. Edited by C. I. MANSUR. Publication of the United States Public Health Service and Tennessee Valley Authority. Pp. i-xiii, 1-422, illustrated. U. S. Government Printing Office, Washington, D. C., 1947.

Principal contributors to this manual were: Mr. C. Kiker, Dr. A. D. Hess, Dr. R. B. Watson, Mr. C. W. Kruse, Dr. R. L. Metcalf and Mr. F. E. Gartrell, of the Tennessee Valley Authority; and Mr. C. I. Mansur, Mr. G. H. Bradley, Mr. R. S. Howard and Dr. C. M. Tarzwell, of the U. S. Public Health Service. The manual was prepared under the general guidance of Dr. E. L. Bishop, Director, Health and Safety Department, Tennessee Valley Authority, and Mr. M. D. Hollis, Sanitary Engineer Director, in charge of malaria control for the U. S. Public Health Service.

An orderly arranged text, which includes 43 tables and 215 figures, describes the development of vegetation favoring anopheline breeding and attendant control methods applicable for antimalarial protection of both urban and isolated rural residents near impoundments in the southern United States. Chapters in the book cover the following general subjects: Malaria and Its Relation to Impounded Water; Planning Malaria Control; Reservoir Preparation; Permanent Marginal Measures; Water Level Management; Shore-Line Maintenance; Larvicide; Mosquito-Proofing; House-Spraying; Facilities and Operation Procedures; Malaria Mosquitoes; Malariology; The Relation of Plants to Mosquito Control; Inter-relationships of Malaria Control and Wildlife Conservation; Personnel Training and Public Relations; Small Reservoirs.

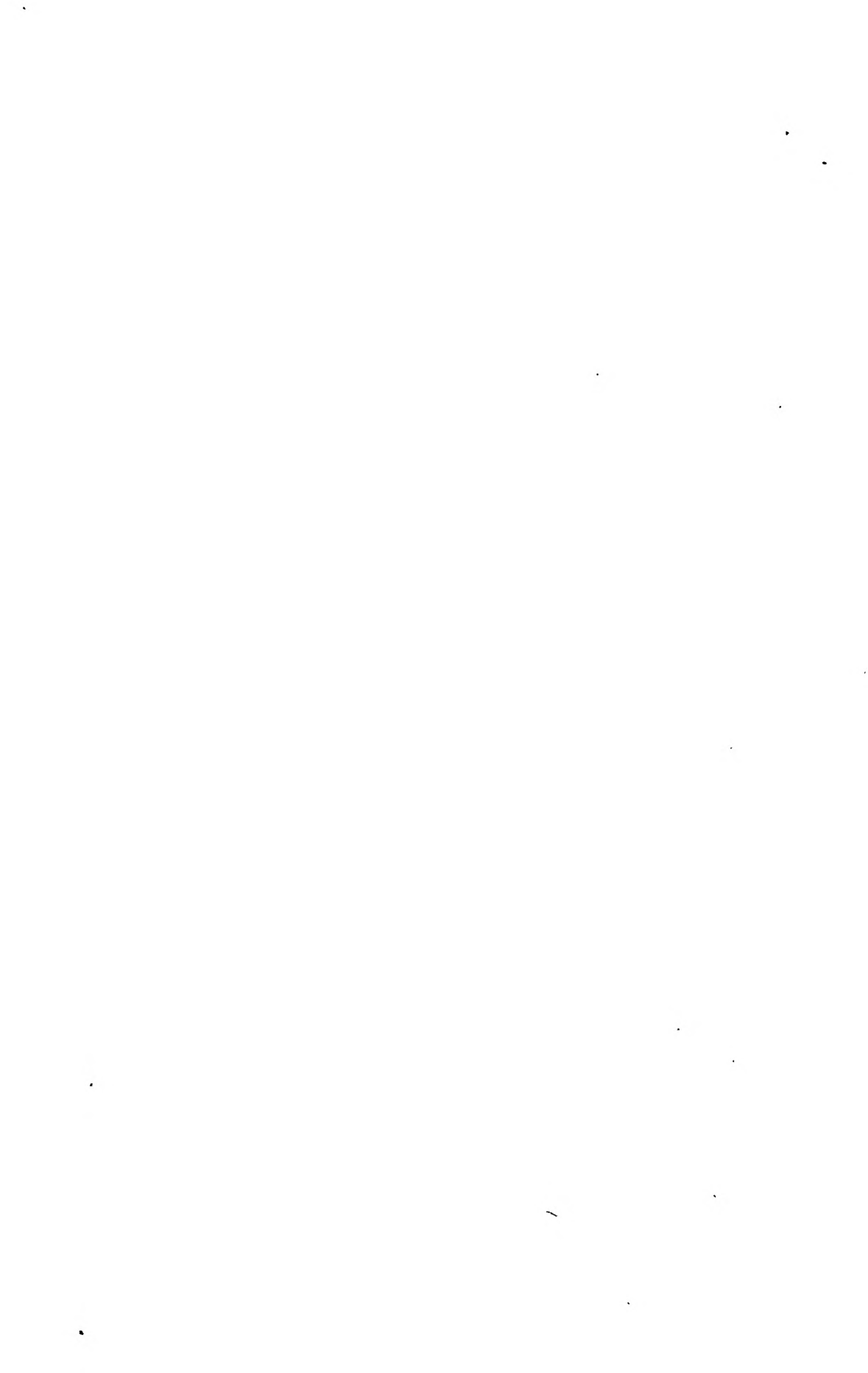
Eight appendices are entitled as follows:

- A. Summary of State Laws and Regulations Pertaining to Malaria Control on Impounded Waters.
- B. Example of Hydraulic Analysis for Prediction of the Frequency and Duration of Flooding in a Flood Control Storage Reservoir.
- C. Chemicals Employed in Malaria Control.
- D. Equipment, Tools, and Supplies.
- E. Design of Airplane Larvicide Equipment.
- F. Methods of Determining the Recovery of Larvicide on a Water Surface.
- G. The More Important Anopheles Mosquitoes Transmitting Malaria in the Principal Regions of the World with Typical Breeding Places and Usually Applicable Control Measures.
- H. Malaria Survey Diagnostic Technique.

The manual presents details of the different subjects with tabulated data covering the necessary tools, supplies and equipment, and comparing the optional measures from the standpoint of initial cost and maintenance. Colored prints of typical water plants help to make the text a clear and outstanding contribution to malaria control literature.

The text advances reasons why endemic centers of malaria in the United States have gradually receded during the past sixty years to the southeastern states. The presence of anophelines without malaria occurs in many areas where malaria formerly existed. Outbreaks of malaria recur in localities as far north as Minnesota, Michigan and Ohio in association with the impounding of water, probably due to infected persons migrating from the South to these areas where they, as well as susceptible, non-immune persons are accessible to biting vector mosquitoes. Therefore, control methods described in this manual have a wide application to impoundments in malaria-free parts of the United States. Much information is given that is of interest to agricultural, welfare and sanitation workers in tropical parts of the world, who may be interested in any one of the methods that reduce the sheltered water areas suited to larvae, destroy larvae, kill adult mosquitoes, or hinder the access to humans by biting anophelines.

E. H. MAGOON



THE PATTERN OF THE LITERATURE OF AMEBIASIS: 1932-1947

A COMMENTARY ON TRENDS^{1,2}

JOSEPH S. D'ANTONI, M.D.³

The late Dr. C. Jeff Miller began his presidential address (1) before the American College of Surgeons by saying, as he tried to thank the Fellows for the honor they had done him, that he found himself lacking completely what Kipling once called "the magic of the necessary word." I hope that you will understand me when I tell you that, finding myself in similar circumstances today, I also find myself lacking that magic. Since this Society was founded 45 years ago, it has held fast to its chief objective, to promote and advance knowledge of tropical medicine. I hope that in my three and one half years of service as its secretary, and during this past year as its president, I have had some small share in the accomplishment of this task. It has been a high privilege to serve this Society, and I am grateful for the honors its membership has bestowed upon me.

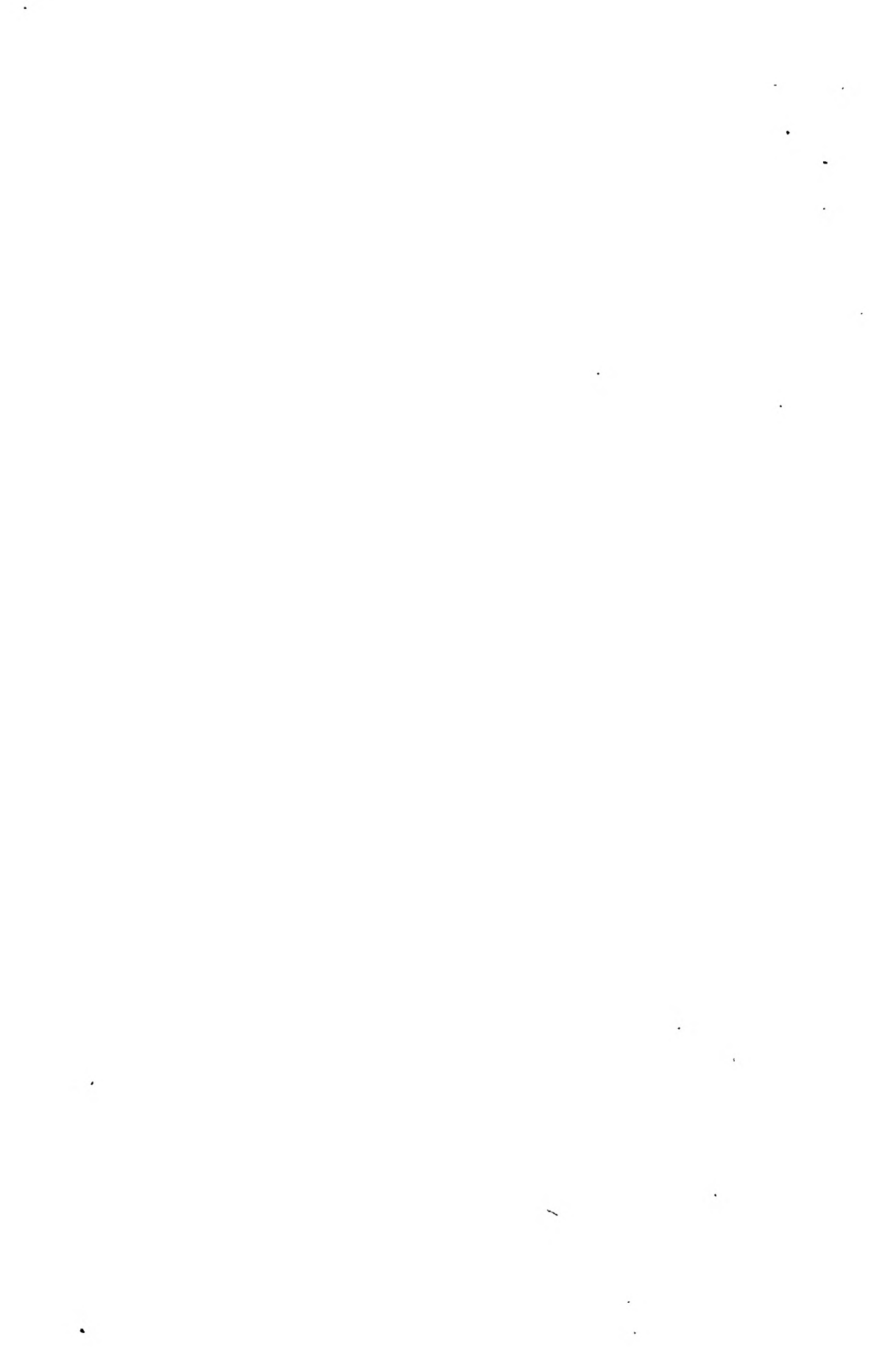
I hope that you will forgive me for opening these remarks on a very personal note. A graduate of the Tulane University of Louisiana who has specialized in tropical medicine could not do otherwise than bear his witness to the teaching which he received in that field and to the men who taught him there. Two of these men, Colonel Charles Franklin Craig, U.S.A., Retired, and Dr. Ernest Carroll Faust, are world authorities in tropical medicine. Because both are former presidents of this Society, and because Colonel Craig for so many years edited its official journal, neither needs any tribute before this audience. Amebiasis, the recent literature of which I have chosen as the subject of my presentation, would indeed have been poorer without their endeavors, and I realized anew, as I prepared these remarks, how much I, like many other graduates of Tulane, owe to their instruction, influence, and wise counsel.

The history of amebiasis as a clinical entity encompasses not quite 75 years (2). It begins in 1875, when Lösch identified the parasite we now know as *Endamoeba histolytica* in a case of dysentery, and reproduced the disease experimentally, though he failed to recognize the cause-and-effect relationship between parasite and disease. The interest of American physicians in the subject, to which numerous contributions had been made during the ensuing 16 years, was first aroused by Councilman and LaFleur, who in 1891 were encouraged by the great Doctor William Osler to report the 14 cases of amebic dysentery which they had observed at Johns Hopkins Hospital. Thus 1891 is an important date so far as the study of tropical disease in this country is concerned. I am not so sure that 1933 is not an equally important date. That was the year, I need not remind you, in which an outbreak of amebic disease in Chicago first turned

¹ From the Department of Tropical Medicine and Public Health, School of Medicine, Tulane University of Louisiana, New Orleans, La.

² Presidential Address, 44th annual meeting of the American Society of Tropical Medicine, New Orleans, December 5-8, 1948.

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the attention of physicians as well as laity to the possible importance of amebiasis in a non-tropical country and to its potentially disastrous consequence.

My own personal interest in amebiasis began with my introduction to it in the Department of Tropical Medicine at the Tulane University School of Medicine, from which I graduated in 1932. In the following year occurred the event to whose importance I have just alluded. I have therefore chosen as the subject of the address which is the duty of all presidents of the American Society of Tropical Medicine, an analysis of the literature of amebiasis from 1932 to July 1, 1947, the latter being the last date for which the *Quarterly Cumulative Index Medicus* is presently available.

MATERIALS AND METHODS

Let me briefly outline the method by which I carried out my study of the literature, as well as the resulting limitations of the investigation.

1. Except for perhaps 75 papers, which for one reason or another I chose to examine in detail, I limited my investigation to the titles listed in the *Quarterly Cumulative Index Medicus* under the headings amebiasis, amebic dysentery and amebic liver abscess. For background, I examined the titles listed under the headings parasites, intestinal parasites, ameba, *Endamoeba histolytica*, and protozoa.

2. This is a superficial analysis in the sense that, with the exceptions just noted, it is based on a study of titles and not of actual contributions. On the other hand, the *Index* editorship is highly competent and its classifications may be assumed to be accurate.

3. Although I have used actual figures, this is not primarily a statistical study. I do not claim complete accuracy for the figures. I made every effort to eliminate the duplicate listings which necessarily appear in the *Index*, but I have no doubt that some duplications have crept in. My aim, however, has been to show trends, and in this respect I think my data may be assumed to be entirely representative.

4. Opinions differ as to the number of medical journals in the world. According to the information kindly supplied me by Dr. George F. Lull (3), Secretary and General Manager of the American Medical Association, the *Lancet* sets the figure at 5,000. The Army Medical Library, which, like the *Lancet*, lists all bulletins in its tabulation, received about 4,000 in 1943. The Editor of the *Journal of the American Medical Association* sets the number in the neighborhood of 2,000. The *Quarterly Cumulative Index Medicus*, the publication of which is by no means the least important task of the American Medical Association, indexed 1,156 journals in the volume covering the period January-June 1947, while the volume for the latter half of 1947, which is now in press, indexes some 60 more. Regardless, however, of the number of journals actually published throughout the world, there is no doubt that the approximately 1,200 journals indexed in the *Quarterly Cumulative Index Medicus* are the most important medical journals published anywhere, and that the roster for the English-speaking countries is essentially complete. I therefore think it fair to say that

this analysis indicates the trends of the literature of amebiasis over the period in question with a high degree of accuracy.

5. For the most part I shall present the results of this investigation graphically, by means of maps and charts, merely commenting on what seem to me to be the most important considerations of distribution and subject presentation.

TEXTS

Over the fifteen and one-half year period covered by this study of the periodical literature, the *Quarterly Cumulative Index Medicus* lists 193 books under tropical medicine and hygiene, and 395 under parasitology and entomology. In this rather appalling total of 588 publications are only 9 titles specially devoted to amebiasis, though the disease is naturally discussed, usually in great detail, in

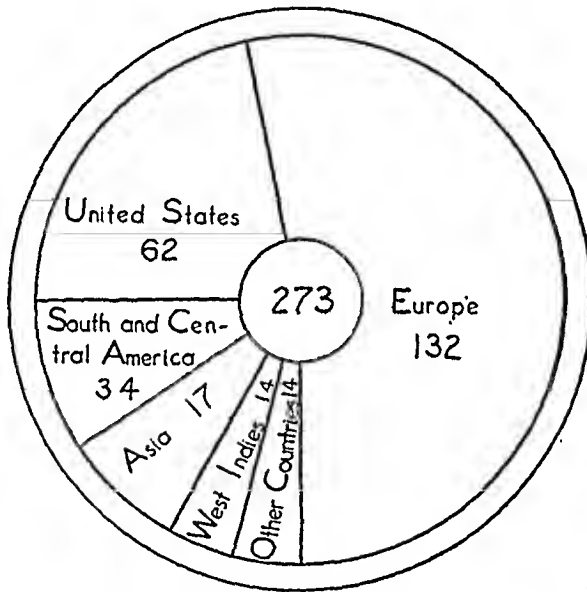


FIG. 1. PROPORTIONATE DISTRIBUTION OF WORLD LITERATURE ON PARASITES (GENERAL) 1932-1947

the large number of texts on tropical disease, protozoa, laboratory methods, parasitology and similar subjects. One of the texts on amebiasis is from Chile, 1 is from Argentina, 1 is from France, 1 is from Japan, and 5 are from the United States. Three of the texts originating in the United States are public health publications, inspired by the Chicago epidemic. The other two books on amebiasis are both by Colonel Craig, *Amebiasis and Amebic Dysentery* (4), which appeared in 1934, and the revision which appeared in 1944 under the title *The Etiology, Diagnosis and Treatment of Amebiasis*. These admirable texts are familiar to all of you and need no further description from me.

BACKGROUND LITERATURE

The background literature for this investigation needs only brief comment. In all of the categories, that is, parasites (Fig. 1), intestinal parasites (Fig. 2),

amebae in general (Fig. 3), *Endamoeba histolytica* (Fig. 4), and protozoa in general (Fig. 5), the major number of articles appeared in European and American journals, the proportion ranging from 63 per cent in intestinal parasites to

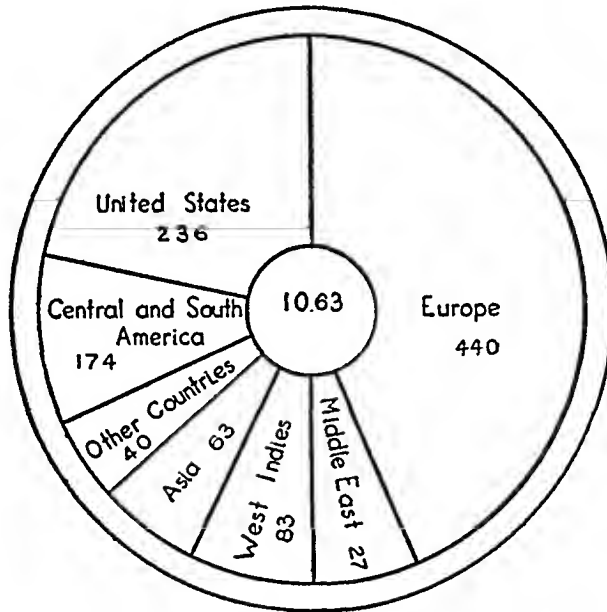


FIG. 2. PROPORTIONATE DISTRIBUTION OF WORLD LITERATURE ON INTESTINAL PARASITES 1932-1947

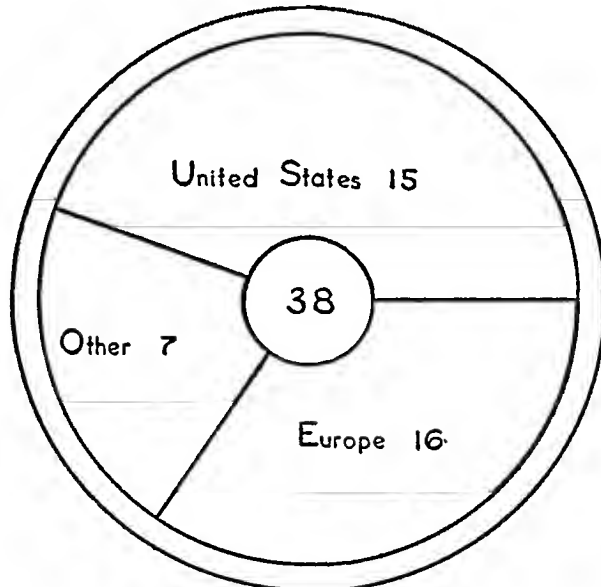


FIG. 3. PROPORTIONATE DISTRIBUTION OF WORLD LITERATURE ON AMEBAE (GENERAL) 1932-1947

83 per cent in protozoa. In all categories except that of *E. histolytica*, the European literature was larger than the American, sometimes considerably larger. This might have been expected, since the majority of the articles published in European journals originated in the countries with colonial possessions,

particularly France, Italy and England. Spain and Portugal were poor seconds. There were 37 articles on intestinal parasites in the Russian literature and 36 in the German literature.

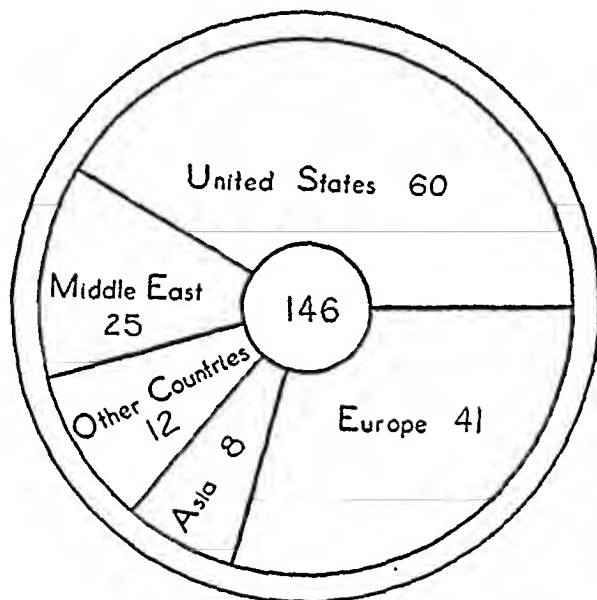


FIG. 4. PROPORTIONATE DISTRIBUTION OF WORLD LITERATURE ON *Endamoeba histolytica* 1932-1947

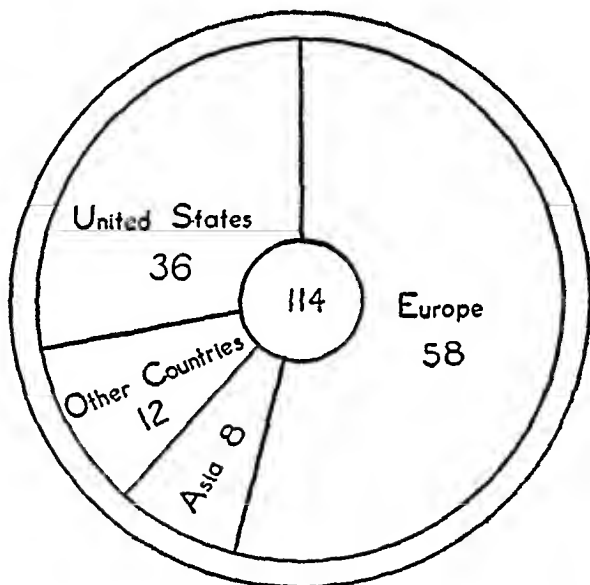


FIG. 5. PROPORTIONATE DISTRIBUTION OF WORLD LITERATURE ON PROTOZOA 1932-1947

The number of published articles from European sources, again as might have been expected, materially diminished during the war years, and while there has been a steady increase in the output from these countries over the last 3 years, the number has not yet reached the pre-war proportions and, in view of the

changes in colonies wrought by the war, perhaps never will. Another comment which ought to be made on this special material is the relatively large number of publications from Palestine, where the *Journal of Oriental Medicine* apparently serves as an organ for several Middle East countries.

GEOGRAPHIC AND YEARLY DISTRIBUTION OF THE PERIODICAL LITERATURE OF AMEBIASIS

Five hundred thirty-four of the 1,500 articles on amebiasis (Fig. 6) published in the world literature between 1932 and July 1, 1947 originated in the United States and the remainder in other countries. The distribution in the foreign literature impresses one, first of all, with the comparative paucity of articles on



FIG. 6. DISTRIBUTION OF WORLD LITERATURE ON AMEBIASIS 1932-1947

this subject in many countries in which amebiasis is a very frequent, and an extremely serious, disease. The majority of foreign articles followed the distribution already mentioned for protozoa, parasites, *Endamoeba histolytica* and other background material: In Europe they chiefly originated in the colonial countries. Elsewhere, Argentina produced the largest number, though again the number from Palestine was surprisingly large.

You will, of course, be interested in the figures for the *American Journal of Tropical Medicine*. Fifty-seven articles on amebiasis, the largest number in any single journal, were published in it during the period in question, 32 before 1939 and 25 during and after that year. The 7 which appeared in 1934 and the 8 in 1935 of course reflected the occurrence of the Chicago epidemic. Between July, 1947, and November, 1948, 6 more articles on amebic infection appeared in the *American Journal of Tropical Medicine*.

It seems significant that in the United States more articles on amebiasis were published in the specialty journals (Fig. 7), including, of course, the *American*

Journal of Tropical Medicine, than in any other type of journal. Unquestionably this tendency is based on the growing realization that amebiasis may indeed affect all parts of the body. I was particularly impressed by the titles listed for the *Archives of Neurology and Psychiatry* in 1935, for the *Journal of Neuropathology and Experimental Neurology* in 1942, and for the *Psychiatric*

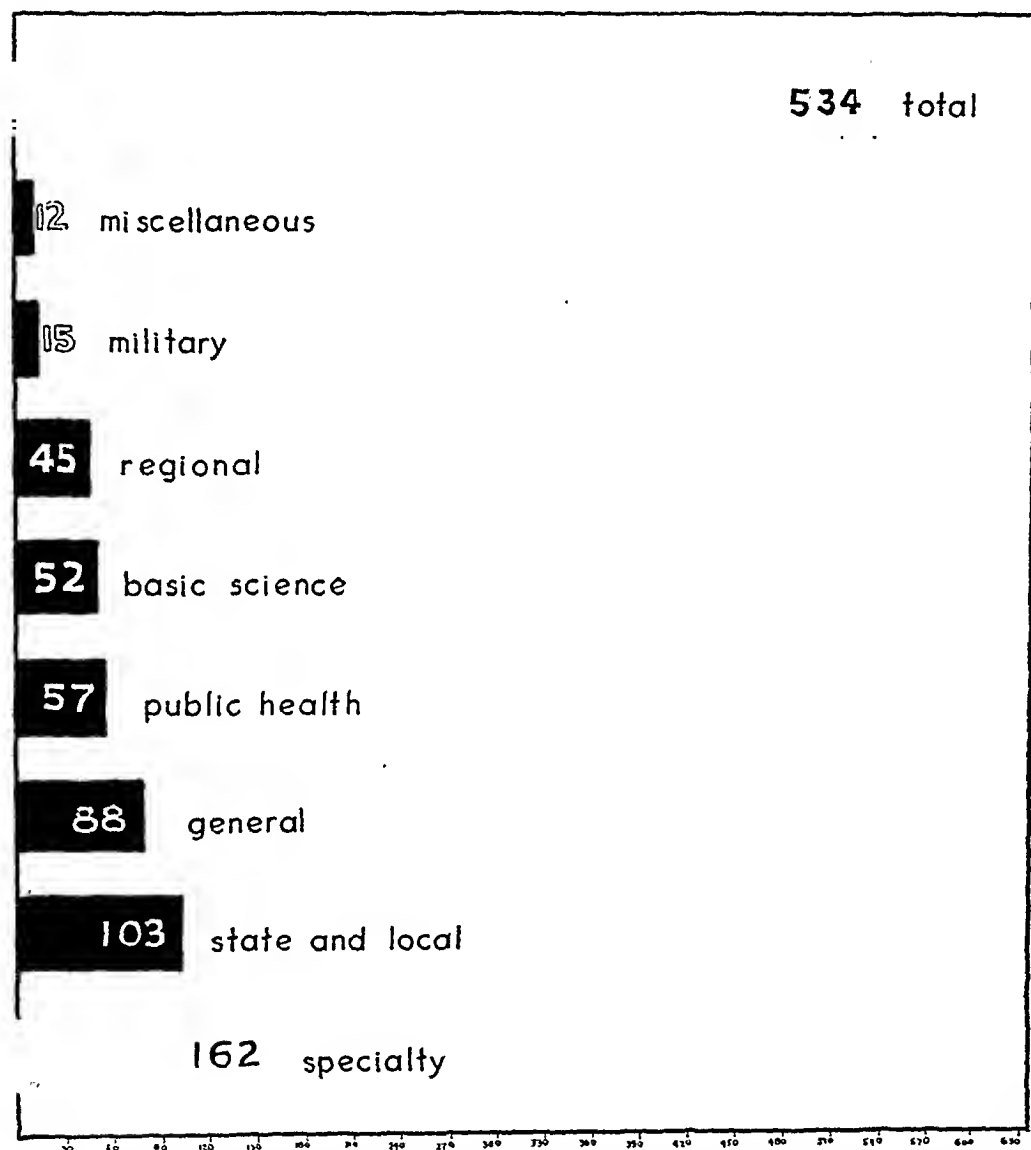


FIG. 7. DISTRIBUTION BY SUBJECT OF UNITED STATES LITERATURE ON AMEBIASIS 1932-1947

Quarterly in 1946, since patients with this disease have so long suffered from the fact that many physicians do not comprehend its neuropsychiatric manifestations, in spite of their prominence in amebiasis. I also find it of interest that articles on amebiasis were published in the *American Journal of Nursing*, the

Bulletin of the American Library Association, Industrial Medicine, Radiology, the Urologic and Cutaneous Review, the Journal of Thoracic Surgery and various general surgical journals.

On the other hand, it is to be regretted, in view of the high rate of infection in the negro race, that only a single article on amebiasis, and that in 1933, was published in the *Journal of the National Medical Association*. It is even more to be regretted that there were only 41 articles published over the entire fifteen and one-half year period in the *Journal of the American Medical Association*.

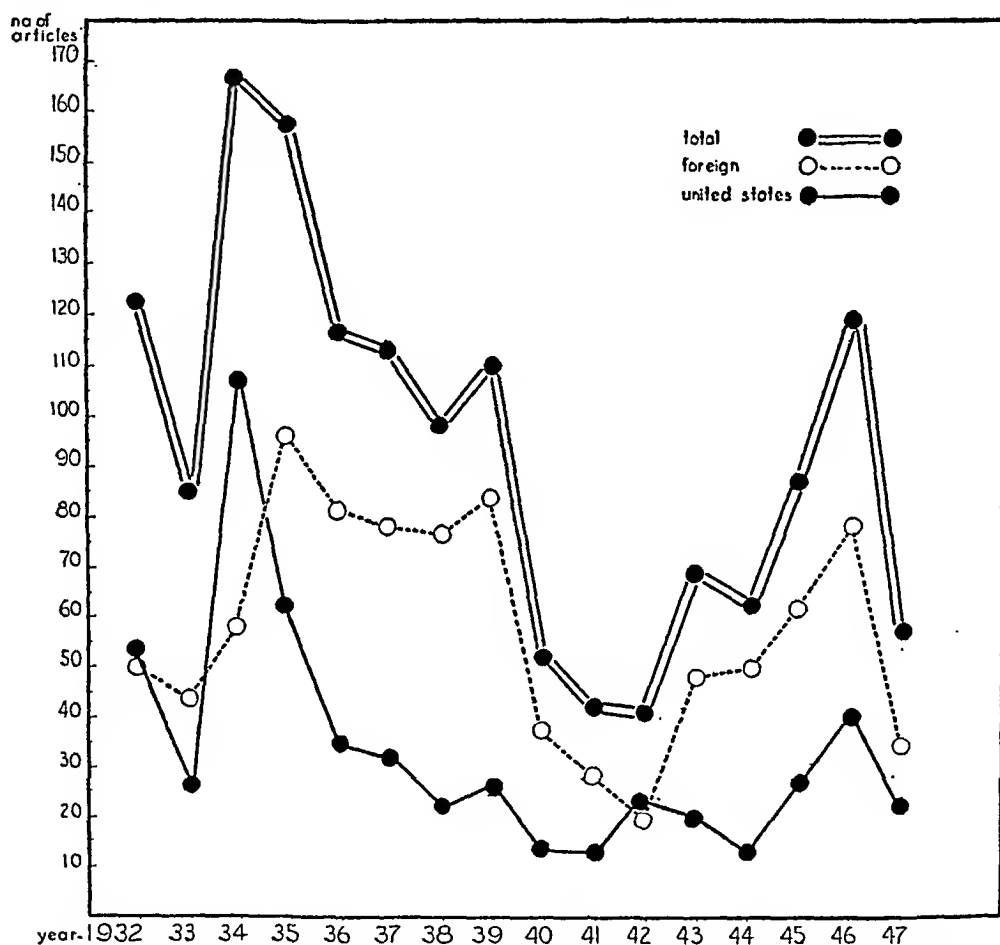


FIG. 8. YEARLY DISTRIBUTION OF WORLD LITERATURE ON AMEBIASIS 1932-1947

ciation. Before pursuing that subject, however, let me speak of the yearly distribution of the articles on amebiasis in the American medical literature and in state and local journals in the United States.

The yearly output of articles on amebiasis was extremely uneven (Fig. 8). The high point was reached in 1934, when 167 were published, and in 1935, when 158 were published. The former was the only year in which the output in the United States literature exceeded the output elsewhere. This fact, as well as the concentration of articles in the 2 years in question, can reasonably be explained by the attention directed to the disease by the Chicago outbreak.

The decided drop in the literature of amebiasis in 1940 and 1941 can be explained, in Europe, by the immediate demands of the war, though the war scarcely explains the drop in the United States output. We did not enter World War II until December, 1941, and in retrospect most of us do not seem to have been especially preoccupied any earlier with the possibility. It is also surprising, in view of the importance of amebic infection in warfare and its possible effects on manpower, to find so small a number of articles on the subject during the actual war years. Since the war there has been an apparent revival of interest in the subject, but whether it will be maintained in the absence of special stimulation it is not possible to say. I might add here that this graph (Fig. 8) and other graphs made up on the same basis are misleading unless it is realized that only the first half of 1947 is represented. Actually, there were 22 articles on amebiasis published in the United States during the first 6 months of

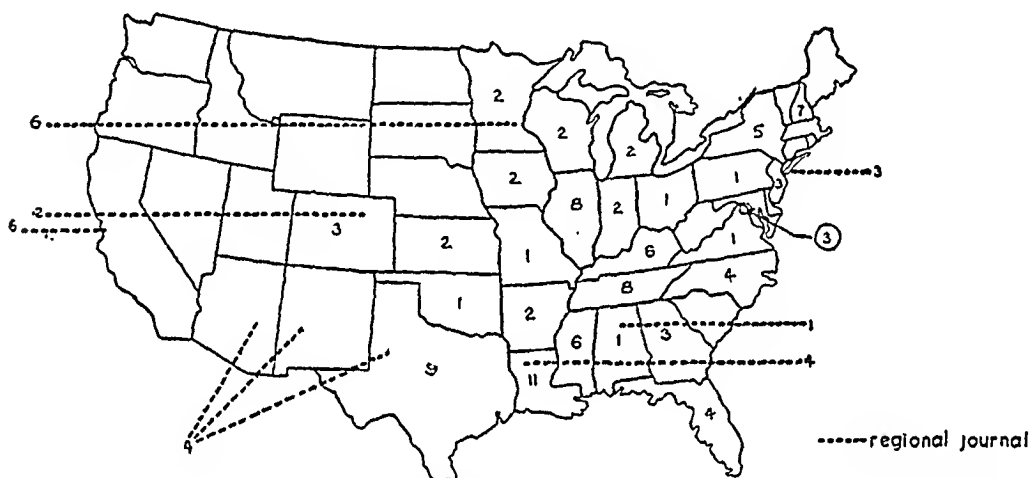


FIG. 9. LOCAL AND REGIONAL DISTRIBUTION OF UNITED STATES LITERATURE ON AMEBIASIS 1932-1947

that year, and if that output is sustained the number will be larger than it has been for several years.

The decreased number of articles following the decline in interest stimulated by the Chicago epidemic was probably to be expected. Three hundred twenty-seven of the 534 articles published over the whole period, 61 per cent, appeared before 1939, leaving only 204 articles to be published over the remaining, longer period of the investigation.

The distribution of articles on amebiasis in the state, local and regional journals in the United States is highly significant (Fig. 9). Of the 126 articles in this category, 100 were published in the 27 state or city journals and 27 in 7 regional journals, such as the *Journal-Lancet* or the *Rocky Mountain Medical Journal*. Twenty-four state journals published 86 articles and 5 city journals published 14 articles. In 2 instances (Dallas and Chicago) the city journals are published in states which have their own state journals, and there is also considerable overlapping in the 5 regional journals which have continued publication to date. A glance at the map will show that in large parts of the country,

particularly parts west of the Mississippi River, no material at all on amebiasis was published in state, local or regional journals during the period in question.

The yearly distribution of state and regional literature was extremely uneven. Sixty of the 100 articles in state and city journals were published before 1939, as were 11 of the 26 articles in the regional journals. Of the 41 articles on amebiasis in the *Journal of the American Medical Association* 32, considerably more than half, appeared before that date. Furthermore, there were no articles in that journal in 1940, 1942, 1943, and 1944, while for the same years there were, respectively, only 5, 4, and 2 articles in state and city journals.

These are tedious details, I grant, but actually they are much more important than they seem. Let us be quite frank. The average physician who is not a

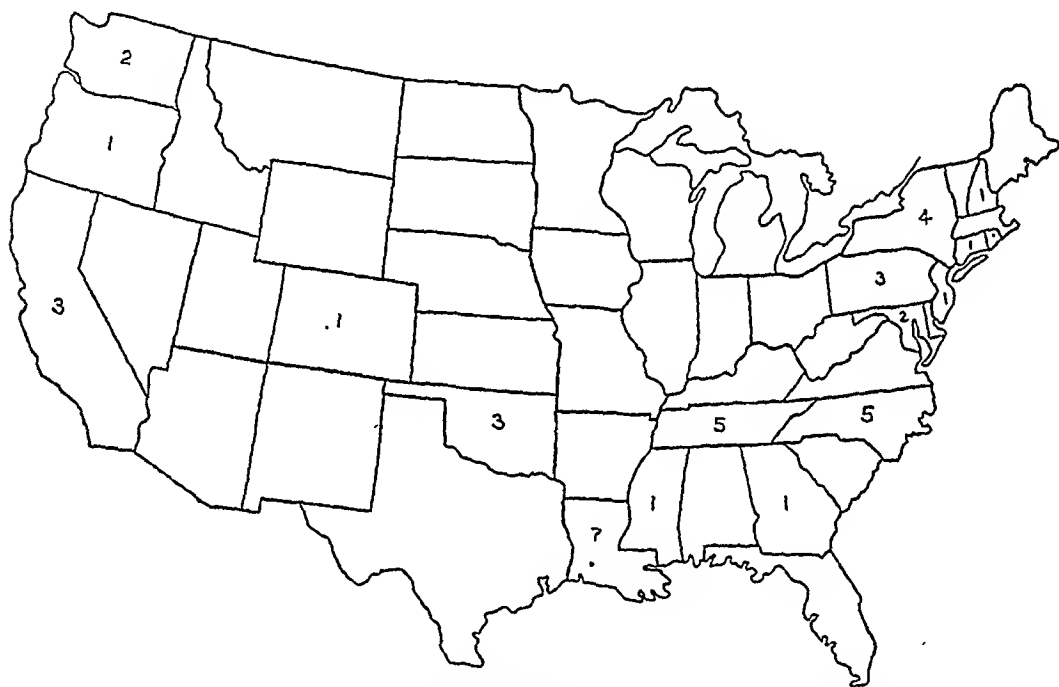


FIG. 10. DISTRIBUTION OF 41 REGIONAL SURVEYS OF AMEBIASIS IN THE UNITED STATES 1932-1947

specialist confines his medical reading, by the kindest estimate, to two to four journals, namely, his state, city or regional journal (sometimes all 3) and the *Journal of the American Medical Association*. The yearly distribution of the literature of amebiasis in those journals, however, makes it quite clear that for a large part of the period surveyed a great many physicians in the United States had no way of securing any current information about one of the most common infectious diseases in the world.

My own opinion is that this observation is perhaps the most important consideration to emerge from the study which I have made. What it amounts to is that after the furor caused by the Chicago epidemic had died away, the physicians of the United States for the most part ceased to be interested enough

in amebiasis to write about it or sufficiently aware of its importance to demand information concerning it.

REGIONAL STUDIES

Of the 41 regional surveys of amebiasis in the United States listed as such in the *Quarterly Cumulative Index Medicus* during the period surveyed (Fig. 10), the majority, 19, were from Southern States. Twenty-five were made before 1939. The largest number in any single state, 7, was made in Louisiana, chiefly in New Orleans, and at that, as I shall point out shortly, we still have no idea of the true incidence of amebiasis in Louisiana or in New Orleans.

Of the 149 foreign surveys (Fig. 11), the largest numbers were made in Europe



FIG. 11. DISTRIBUTION OF 149 REGIONAL SURVEYS OF AMEBIASIS 1932-1947

and South America, though 7 were made in Canada and 18 in Africa. It should be noted that the region of the survey and the place of the published report did not always coincide. In 1937 a survey from Eritrea was published in an Italian journal while in the same year a study from Armenia was published in a Russian journal. Studies from Venice in 1944 were of German origin but another study from Italy in 1946 was published in a British journal. Studies from India appeared in British journals and in *Gastroenterology* and the *Annals of Internal Medicine*. A 1942 study from the Middle East concerned troops from South Africa, while a 1947 study from that region appeared in the *Archives of Internal Medicine*. In 1946 a study of amebiasis in Northern Ireland appeared in the *Annals of Internal Medicine*. For the most part these geographic discrepancies seem to reflect the (changing) fortunes of war and are not to be related to the colonial interests previously mentioned as explaining the preponderance of articles from certain European countries.

SUBJECT DISTRIBUTION OF THE LITERATURE OF AMEBIASIS

For obvious reasons the presentation of the subject distribution of the literature of amebiasis is necessarily very general (Fig. 12), but the categories which have been selected indicate fairly well the proportionate distribution of the interests of the writers on this subject during the fifteen and one-half year period surveyed. Brief comments might be made on certain special subjects.

The Chicago Epidemic. Sixteen articles in the literature in the early period of this survey specifically concern the Chicago outbreak, and it would be impossible to say how many of the large number of articles which appeared in the next few years took their origin from it. Attention has already been called to the fact

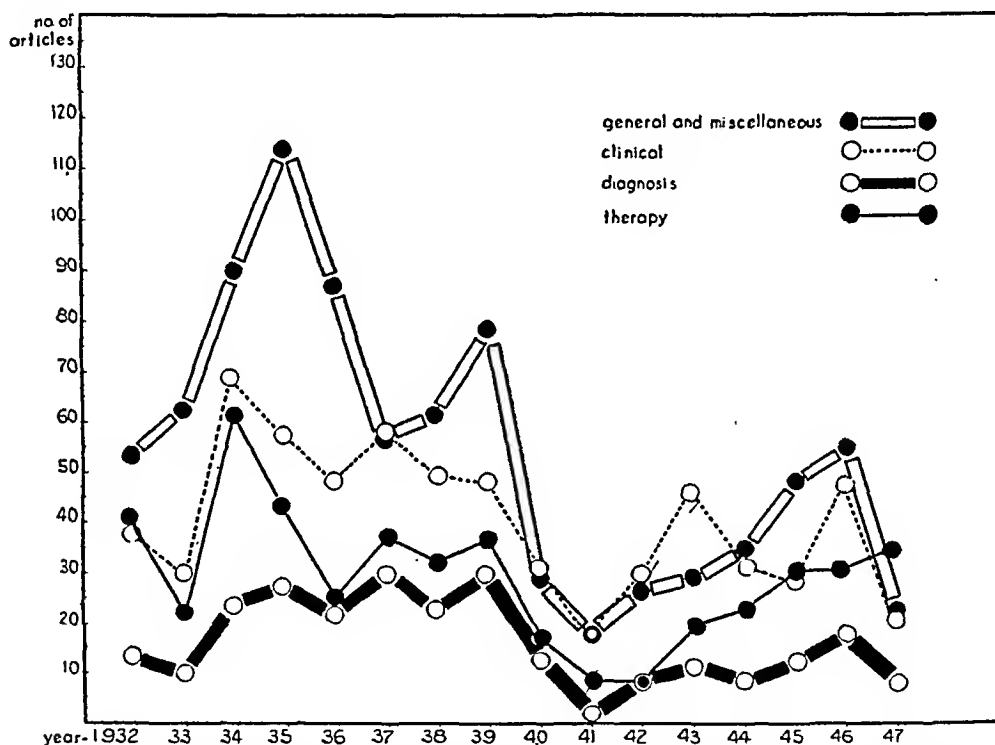


FIG. 12. YEARLY DISTRIBUTION BY SUBJECT OF WORLD LITERATURE ON AMEBIASIS 1932-1947

that the highest quantitative point of the foreign and United States material on amebiasis was reached in the 1934-1936 period and was unquestionably the result of the Chicago experience. The repercussions were, in fact, enormous. The material in the *Journal of the American Medical Association* included special reports, a comprehensive review (5), and a great deal of quite animated correspondence. There was an exhibit on amebiasis at the Cleveland session in 1934 and one of the general scientific sessions was devoted to this disease.

A re-reading of the reports on this epidemic recalls some interesting facts and gives rise to a good deal of thought. At this distance in time it seems even more fortunate than it seemed when it happened, that the first cases were diagnosed and that their significance was realized without too much delay. The

results were tragic enough, but otherwise they would certainly have been more terrible. By January of 1934, you may remember, more than 700 cases had been identified in 206 cities in all parts of the country, as well as in Canada and in Paris, and 14 deaths had occurred in Chicago alone (6). Eventually cases were identified in more than 400 cities in 43 states, 3 Canadian provinces, and Hawaii. In all, 1,409 known cases of amebic dysentery resulted in 98 deaths (7).

In their first reports on the Chicago epidemic, Tonney and his associates (8) virtually ruled out milk and water as responsible for the infection. It was therefore ironic that polluted water, or, more correctly, pollution of the water supply, was soon afterward proved to be the chief cause of the outbreak. In fact, as many writers have pointed out, the whole situation in Chicago was ironic. Pollution from water is usually suspected only in rural communities, where wells are adjacent to homes, not in modern hotels in large cities.

One might expect, in view of these findings, that numerous articles would be written on this unsuspected source of amebic infection. A 1936 report, in fact, emphasized the importance of routine inspections of plumbing installations (9). The lead does not seem to have been followed up in the medical literature. The only (3) articles on plumbing, water-transmission and sewage-transmission appeared in 1935 and 1936. Nothing else appears in the literature under those specific headings. This is unfortunate, since the literature not usually available to physicians makes the possibilities of water-borne outbreaks of disease, even in this day, nothing short of alarming (10, 11).

The 2 medicolegal articles which appeared in 1935 and the 1 which appeared in 1939 were of course echoes of the Chicago epidemic.

Food-Handlers. The large number of food-handlers in the involved Chicago hotels who were found to have active amebic infections or to be carriers of the parasite naturally pointed the finger of suspicion at this source, which, in the first phases of the investigation, seemed extremely important (8). The fact that 1 of the carriers had also been implicated in the minor outbreak studied in 1927 in another Chicago hotel by Kaplan and her associates (12) seemed to strengthen the conclusion. Colonel Craig has always laid considerable emphasis on this possible source, but others are less enthusiastic. McCoy and Chesley (13) deprecated it in their 1934 report and pointed out the complete impracticability of examining this type of personnel. Food-handlers, they emphasized, include much larger numbers of persons than those who work in the dining room and kitchen. Moreover, the number of personnel limited even to these parts of hotels makes their examination an unrealistic proposal. In the strictest sense there were in Chicago in 1934 12,000 food-handlers. Even if each one had been submitted to 3 stool examinations, a quarter to a third of the infections would have remained undetected, these authors point out, while the task would have occupied 4 years if single specimens had been examined and some 12 years if the task had been carried out properly. In the face of figures such as these, the proposition is reduced to complete absurdity.

In connection with food-handlers as sources of amebic infection, the point made by Andrews (14) in 1933 was well taken, that the mother is the food-

handler in the family, so that attention to her status would do much to reduce the family incidence of amebiasis.

Our own experience with amebiasis in childhood has convinced us that most children with the disease become infected during the first 2 years of life, and that the most reasonable explanation of the disease at this age is mother-to-child transmission. During this period of life, the mother (or nurse or whoever is responsible for the child) is in actual physical contact with him a good part of the time, feeding, bathing, dressing and changing him, teaching him to walk, or merely fondling him. In our cases in young children we almost invariably find that the individual caring for the infected child is herself infected and is often passing cysts in such numbers that diagnosis can be made on the first stool examination.

Familial amebiasis is so common that one would expect to find numerous articles on this phase of the disease instead of the few published on the subject, all before 1940. In this same connection, Ikeda (15), in one of the numerous reports on groups of patients who contracted their infection in Chicago, pointed out that 2 of his 9 patients (all from St. Paul) were in the same family and that all 9 were of the upper social strata, where one would expect personal hygiene to be beyond reproach.

Incidence. When Craig (16) stated in 1927 that from 5 to 10 per cent of the population of the United States was infected with the parasite *E. histolytica* his remarks, as you know, were greeted with a good deal of skepticism. Among those who know the facts I have heard no adverse comment on his statement, in 1944 (2), that the return of troops from heavily (and seriously) infected regions would greatly add to the conservative 10 per cent estimate of such infections in this country. If Mackie's (17) studies at the Tropical Disease Clinic of the Veterans Administration in Winston Salem, North Carolina, can be accepted as typical, the incidence in some veteran groups is 40 per cent. The figure is based on 330 adequately studied veterans and the series is, of course, selective: These men reached the center because they were suspected of having the disease, though suspected, it should be added, only after they had made the rounds of other physicians and other hospitals, by whom and in which they had been inadequately studied and incorrectly treated.

As a matter of fact, we have no idea of what the true incidence of amebiasis in the United States is, and this investigation certainly throws no light on the subject. The most representative investigation during the period was probably that carried out by Faust (18), in 1941, on 202 accident victims submitted to autopsy at Charity Hospital of Louisiana at New Orleans, but the number of cases is too small to be representative.

I might pause here to reiterate what you know very well, that the reports of city and state departments of health are manifestly inadequate. Amebiasis is a reportable disease in most states, but I beg leave to doubt that it is reported any more fully in most states than it is in Louisiana. The New Orleans Board of Health report (19) from January 1, 1932, to November 23, 1948, shows no cases at all in 1932 and 1933, and a total of 656 for the remainder of the period, the

number ranging from 2 in 1940 and in 1941 to 162 in 1946. The state statistics (20), which run from 1933 through July 1, 1947, show a total of 1,428 cases, the number ranging from 32 in 1941 to 188 for the first 6 months of 1947. These statistics are manifestly absurd. I have seen as many patients in private practice myself during the last 2 or 3 years. I am inclined to believe that the most interesting thing about these reports is that in 1941 the nomenclature was changed from amebic dysentery to amebiasis.

A properly conducted representative survey of amebiasis would obviously introduce enormous and perhaps insuperable difficulties, if only because it would require an amount of cooperation from the subjects quite unlikely to be received. Most of the reported incidences of amebiasis seem to be based on single-stool examinations, and such examinations, as Dobell (21), in 1917, was the first to point out, discloses not more than a third of the actual infections in any locality surveyed.

The full implications of that estimate open wide possibilities. According to Faust (22), writing in 1942, if several examinations were made by improved methods in each patient studied, the general United States incidence of amebiasis might well prove to be 20 per cent, while in poorly sanitized regions of the Western Hemisphere, particularly moist tropical regions, an incidence of 50 to 95 per cent might be "possibly a reasonable estimate." I believe that one of the responsibilities of the membership of this Society is to hammer home that possibility.

Clinical Manifestations. The clinical manifestations of amebiasis were the predominant theme of 656 articles in the world literature (Fig. 12). A mere reading of the titles makes clear at least three facts:

1. Amebiasis affects every part of the body. Special articles deal with the disease in all portions of the gastrointestinal tract including the gums and other parts of the mouth, the heart, the genitourinary system, all parts of the thorax, the liver, the biliary tract, the spleen, the muscles, the nervous system, the eye and the brain. Manifestations were frequently multiple. I imagine that this composite list would furnish some surprises to physicians who still think of amebic infection as limited to the intestinal tract and the liver.

2. Amebiasis can produce almost every conceivable single symptom as well as many syndromes. Special articles deal with such manifestations as neuropsychiatric symptoms, ameboma, hemorrhagic intestinal manifestations, intestinal obstruction, intestinal perforation, jaundice, false angina, a pellagra-like syndrome, a pseudo-carcinomatous syndrome and a pseudo-tuberculous syndrome, to mention only a few.

In this connection, an extremely interesting article was written by Boyers (23) in 1933. From the histories of 700 patients with amebiasis he was able to tabulate 1,961 separate complaints, referable to the gastrointestinal tract in 29 per cent, to the neuromuscular system in about 25 per cent, and to the bones and joints in about 13.6 per cent. Appetite, digestion, bowel habit, respiratory and cardiovascular function, urinary function, sexual status, the skin and mucocutaneous areas, the glandular system, memory and morale were all affected.

A significant fact was that the wording of the complaints in many instances unquestionably reflected the opinion of physicians who had previously been consulted and who, by inference, had failed to make the diagnosis. Boyers' conclusion is nothing more than commonsense, that unless physicians learn the possible manifestations of the disease, the problem of chronic amebiasis in temperate and subtropical zones will not be solved.

3. Amebiasis frequently appears in combination with other conditions. Case reports concern its association with peptic ulcer (ruptured and unruptured), pregnancy and the puerperium, carcinoma and polyposis of the colon and rectum, intestinal fistula, perinephric abscess, typhoid, paratyphoid, blackwater fever, suprarenal insufficiency, and phlebitis, as well as balantidiasis, lambliasis and bacillary dysentery. It is interesting to note that in the review of amebiasis (unfortunately entitled "Amebic Dysentery") published in the *Journal of the American Medical Association* (5) shortly after the outbreak of the Chicago epidemic, the possibility of the association of amebiasis and bacillary dysentery was mentioned, though there was no apparent realization, as there still is no general realization today, that both diseases must be treated if a cure of amebiasis is to be effected.

Much of the clinical literature still indicates a rather naive surprise that serious amebic infection can exist in the absence of clearcut clinical symptoms, though Craig and others have been stressing that fact for many years. In some of his earliest writings Craig noted that ulceration of the intestinal tract and amebic liver abscess can exist without a history of parasitic infection and without dysenteric symptoms, yet numerous titles still indicate by their actual wording the writers' amazement that these things should be. Other titles have to do with atypical manifestations of the disease, though the comment seems warranted that whether the term atypical should be used depends entirely upon one's concept of the disease and one's knowledge of it.

Diagnosis. One rather surprising aspect of this study was the relatively small number of articles on diagnosis (Fig. 12), particularly in 1941, when a single title was listed on this subject. Since no conclusions worth anything can be drawn concerning the incidence of amebiasis, and since no treatment can be instituted, until the diagnosis is made, I should be inclined to say that the lack of emphasis on this phase of the subject has much to do with the unsatisfactory status of amebiasis at the present time.

Diagnosis in the Chicago epidemic was of course bad, the chief reason for the serious diagnostic error being the usual one, that no one thought of amebiasis.

Twenty-eight of the 53 articles on laboratory diagnosis appeared before 1939. Of the 17 articles on sigmoidoscopy, 10 appeared between 1943 and 1947, a hopeful sign to those of us who emphasize this method of diagnosis. Twenty articles under the heading of diagnosis concern the complement-fixation test, 4 being by Craig himself. I continue to believe that the reason that the general results with this test do not equal those secured by Craig is that his instructions are seldom followed accurately. Seven of the 17 articles on X-ray diagnosis appeared between 1944 and 1947, and the other 10 between 1936 and 1939.

We have naturally been gratified with the reception accorded the zinc sulfate technique, which we introduced in 1937, and with its rather general usage. We regret, however, that with the exception of Baroddy's modification, this technique has apparently not been improved, and we also regret that a satisfactory non-etiological test has not been introduced. Many of the nonstandard tests described in the literature under survey were mentioned once and then appeared no more. The urine test, the tissue identification method, the emetine therapeutic test, the Takata test, congelation reactions and the Weltman coagulation test were for the most part described between 1935 and 1939.

An occasional article under the heading of diagnosis presents a curious point of view. Thus Huston (24), in 1937, pointed out the usefulness of a study of the leukocytes in the diagnosis of amebiasis and recommended a therapeutic test with emetine for the same purpose. Examination of the stools, he remarked somewhat complacently, while a useful method, "has its limitations." Even Doughty (25), one of the few writers to emphasize the incidence of amebiasis in general practice, stated that diagnosis is not difficult. The explanation of his good results seems to lie in the fact that he examined purgative stools and intestinal mucus and repeated his examinations; he was usually successful with the third specimen.

Therapy. The 469 articles which deal with therapy (Fig. 12) cover a wide field. The literature is characteristic: An enthusiastic report on some agent or method is followed either by dwindling interest or complete silence. The bulk of the articles concerned with special drugs dealt with emetine (50), iodine compounds (37), the arsenicals (28), the sulfa drugs and penicillin (12) and some 29 other different drugs or combinations of new or old drugs. Most of the items in the latter category were listed only once.

In addition to articles on drug therapy, are the articles concerned with such special methods as autovaccination, rectal therapy, hydromineral and thermal methods, typhoid vaccination, and, perhaps most curious of all, the oxygenation treatment with which Golob (26), in 1936, reported the cure of a patient on the point of death from amebic infection.

Of the 11 articles dealing with reactions from specific drugs, 6 dealt with emetine, 1 with arsenic and emetine, and the remainder with arsenicals. Our own experience with emetine continues to indicate that it is a highly dangerous drug unless it is given in smaller quantities than are usually regarded as safe and with far more elaborate precautions than are usually considered necessary.

My own experience with the therapy of amebiasis is probably typical. I was originally extremely enthusiastic about diodoquin. Now I know that it is not the solution of the therapeutic problem in this disease. Over the past 18 months I have used 8 new amebicidal drugs, all adequately tested in respect to toxicity and all submitted to a reasonable clinical trial. They include an organic iodide, a sulfonamide, an antibiotic, an organic chemical compound, and 4 arsenicals. Perhaps among them lies the answer to our problem, but I shall wait awhile before I express myself in print on the subject.

The 28 articles on the surgical considerations of amebiasis were distributed

fairly evenly over the fifteen and one-half year period. The number clearly indicates the widening field of therapy in amebiasis. These articles also reiterate the extreme harm which can be done by ill-advised (that is, non-urgent) surgery in a recognized amebic infection and the disastrous consequences which can follow surgery in an unrecognized infection. The latter was one of the most appalling aspects of the Chicago experience.

A casual survey of numerous articles written during this period has convinced me of the need for revision of our standards of cure. We must continue to emphasize, of course, that no cure can be pronounced without laboratory evidence, but I would judge from these articles that we must also emphasize the necessity for *complete* relief of clinical symptoms. The insidious nature of the disease is responsible for this anomalous situation. Patients who have been ill for a long time with amebiasis, or who have been infected since childhood, react with gratitude to any significant amelioration of their symptoms. We must not be content with this partial response but must emphasize that a clinical cure of the patient with amebiasis uncomplicated by any other disease implies a complete state of well-being, not merely an improved status.

The introduction of the zinc sulfate technique in 1937 promptly showed that the drugs then in use, some of which had been thought to be 90 per cent effective, were actually considerably less effective. This was because the enriched method permitted the recovery of larger numbers of cysts and thus the identification of lighter infections.

Recently we have encountered another interesting diagnostic-therapeutic relationship. In addition to the fact that certain strains of *E. histolytica* are apparently more readily affected by certain drugs than by others, we have discovered that therapeutic efficiency greatly increases the difficulty of post-therapeutic testing. If the amebicide administered is effective, the number of cysts in the stools is depressed and the superiority of purgative specimens over normally passed stools practically disappears. This is true even in patients who before treatment had had numerous parasites in purgative specimens and in aspirated material. In these cases even sigmoidoscopic examination must be repeated one or more times for reliable results after treatment. Unless this precaution is taken, the patient may be erroneously classified as cured and will learn that he is not, only when an intercurrent infection or some other cause again lowers his resistance and brings about a return of symptoms.

If this observation is confirmed by other physicians, it will be important: A single purgative specimen is now regarded as equal, for diagnostic purposes, to 3 normally passed stools. It may be that when amebicides are effective this ratio no longer holds and that the negative report on a purgative specimen is as unreliable as a negative report on normally passed stools.

LIVER ABSCESS

The geographic distribution of the world literature on amebic liver abscess (Fig. 13) shows that the majority of articles originated in the United States and Europe. The preponderance in Europe is again in the colonial countries,



FIG. 13. DISTRIBUTION OF WORLD LITERATURE ON AMEBIC LIVER ABSCESS 1932-1947

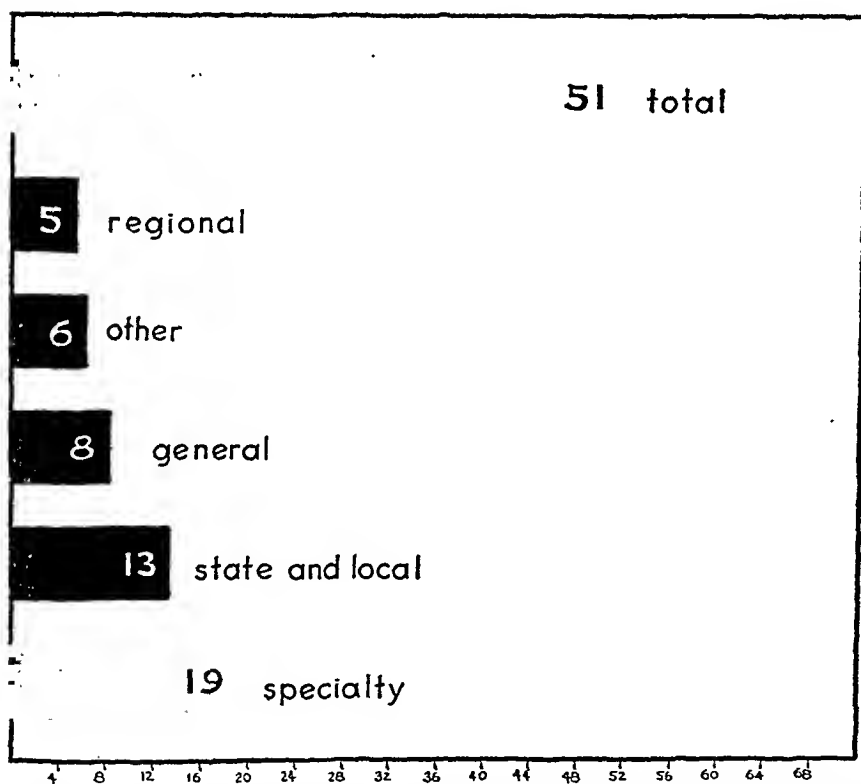


FIG. 14. DISTRIBUTION OF UNITED STATES LITERATURE ON AMEBIC LIVER ABSCESS 1932-1947

England, France and Italy, which accounted for 63 of the 75 articles from that area. Of the 42 articles in South American journals, 24 were from Argentina, this being a larger number than from any other country except France and the United States. Other than Argentina, however, no South or Central American country produced a significant number of contributions. As might have been expected, the number of articles in European journals decreased during the war, while those from South America rose proportionately.

In the United States (Fig. 14), a considerably larger number of articles on amebic liver abscess appeared in the specialty journals than in the general journals (8) or in state and regional journals combined (17). In one sense this very uneven distribution is easy to understand, since amebic liver abscess is a condition which demands highly specialized treatment. It represents, however, another unfortunate trend: Amebic liver abscess is a condition with which the general practitioner should be familiar. Its manifestations often in no way suggest the origin of the symptoms, with the result that diagnosis is missed in a regrettably large number of cases. My own experience, I am sure, is duplicated by that of other physicians who practice our specialty: Not infrequently I hear of, or witness, autopsies in which completely unsuspected amebic liver abscesses are brought to light.

The subject-distribution of the world literature of amebic liver abscess (Fig. 15) bears out the pessimistic comment I have just made. One of the largest categories concerned with this complication of amebiasis had to do with rupture, which is a sad commentary on our diagnostic acumen. The 37 articles in this category deal with rupture of amebic liver abscesses in general and with rupture into the thoracic cavity, the lung, the pleura, the bronchus, the stomach, the pericardium and the peritoneum in particular. The majority of these articles, 22 of the 37, were written before 1939, it is true, and it may be that the situation has permanently improved. I wonder again, however, whether a possible explanation of the attention devoted to these catastrophes, which are recorded chiefly in the form of case reports (the percentages, of course, are overlapping) might not lie in the small amount of attention devoted to diagnosis: 17 of 204 articles in the world literature on amebic liver abscess devoted to the important subject of diagnosis seem a small number over a fifteen and one-half year period.

OTHER CONSIDERATIONS

Amebiasis in Children. Over the past 2 years I have treated or seen in consultation approximately 400 children under 12 years of age, in all of whom amebic infection was confirmed by laboratory methods. This experience is, of course, in contrast to the general idea that amebiasis is uncommon at this period of life, though it bears out the maxim all of us quote, to the effect that this disease will be found wherever it is looked for. At first glance the 33 articles on amebiasis in children in the world literature between 1932 and 1947 might suggest that there is a general realization that the disease is not uncommon at this age. Actually, the reverse is true. Most of the articles are merely single case reports, or reports of 2 or 3 cases, which have apparently been published because of the belief that the condition is very unusual in children.

Military Aspects of Amebiasis. Of the 35 articles on the military aspects of amebiasis during the period surveyed, 28 were published after 1935, which is understandable, since it was about at this time that Italy's advance into Ethiopia set off in Europe the spark which Japan had already lighted in the Orient by her Manchurian adventure. These 35 articles are exclusive of 3 reports from the Mediterranean and 1 from the European Theater of Operations, which I omitted because of the special circumstances under which they were prepared.

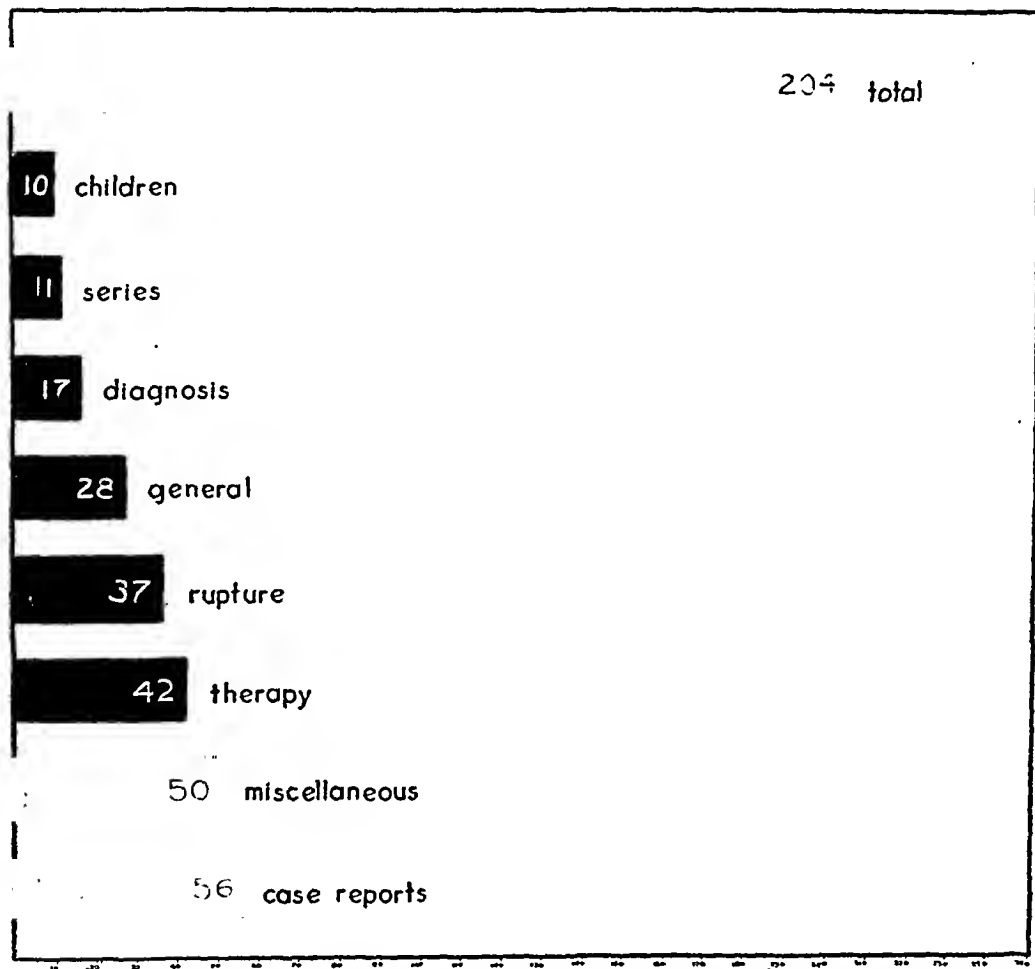


FIG. 15. DISTRIBUTION BY SUBJECT OF WORLD LITERATURE ON AMEBIC LIVER ABSCESS 1932-1947

Insurance in Amebiasis. I have long felt that the patient with amebiasis is the forgotten man so far as insurance is concerned. I have full sympathy with the insurance companies in their desire to protect their other clients, and, of course, to protect themselves, but I also cannot understand why the physicians who serve these companies cannot make clear to the men who set the rates and determine the policies (1) that a patient with a history of amebiasis who has been properly treated, or a patient who is under treatment at the time of application,

is highly unlikely to develop any serious complication, particularly hepatitis or liver abscess or both; (2) that many of the patients they are insuring without question from this standpoint may harbor the infection; and (3) that the report of a single negative stool is not worth the paper it is written on. On second thought, however, I can understand the situation which now exists: The majority of insurance physicians know no more of the facts of amebiasis than do the majority of other physicians. It was a matter of interest to me that the single article on this phase of amebiasis in the literature during the period surveyed, by Cawston (27), in the *Practitioner* in 1940, expresses much the same point of view as my own, while the paucity of the literature helps to explain the point of view of the insurance companies.

The Curious Case of Dr. O. Ublavici. You will probably remember that the literature of this period contains Dobell's (28) exposé of the curious case of Dr. O. Ublavici. In 1887 the Professor of Pathologic Anatomy at the University of Prague, a certain Jaroslav Hlava, published in the *Journal of Czech Physicians* an article entitled "O. Úblavici." which means "on dysentery." In it he reported the isolation of *E. histolytica* in the stools of patients with dysentery and the transfer of the infection to cats. The latter was then an extremely important demonstration, though whether because of it Professor Hlava should be ranked with Koch and Pasteur, in whose ranks Manwaring (29) placed him in 1939, is perhaps open to question. At any rate, the article was abstracted by Kartulis, who by some aberration listed the author as O. Ublavici. And as O. Ublavici he continued to appear in the literature, even receiving doctoral honors in an *Index Catalogue of Medical and Veterinary Zoology*, until Dobell, in 1938 cleared up the confusion and restored the professor's personality to him.

Nomenclature. One of Craig's (30) greatest services to amebiasis was the paper which he wrote in 1921, in which he outlined the modern concept of amebiasis. This concept, I need not remind you, classified amebic dysentery as merely a phase of the disease known as amebiasis. Craig pointed out then, almost for the first time, that dysentery is a comparatively uncommon, though by no means rare, manifestation of amebic infection in nontropical countries and that diarrheas and other symptoms of gastrointestinal irritation are very commonly present in carriers of *E. histolytica*, even though these persons may regard themselves as in good health. For every single case of dysentery in the United States, Craig continued, there are hundreds of cases of diarrhea caused by this disease and still larger numbers of cases of constipation and other gastrointestinal disorders.

How slow the recognition of these truths has been is well demonstrated by the fact that it was not until July, 1933 that the *Quarterly Cumulative Index Medicus* ceased to index amebic dysentery as if it were a separate disease, and placed it instead, where of course it belongs, under the heading of amebiasis, along with other manifestations and phases of this disease. The change in nomenclature seems to have resulted in a change of emphasis on the part of writers on amebiasis: During the one and one-half years in which amebic dysentery was indexed as amebic dysentery, there are 63 references to it, against only 145 over the remaining fourteen years of the period surveyed.

The first comment which comes to mind in connection with amebic liver abscess is along the same lines, the hope that eventually the editors of the *Index* will see fit to include it, just as they have included amebic dysentery, under the heading of amebiasis, thus bringing together under a single classification all the manifestations and phases of this disease. The present classification is not logical, particularly since nonsuppurative hepatic conditions on an amebic basis are listed under amebiasis. This is, of course, a small point, but a physician unfamiliar with amebiasis might easily, if unwittingly, derive from the present arrangement the impression that he was dealing with two separate conditions.

THE PROBLEM OF AMEBIASIS

In his text on amebiasis published in 1944 Craig (2) commented on "the surprising amount of ignorance regarding this infection among many physicians" and attributed it, "as in the past, to the general belief that amebiasis is a tropical infection, of little interest to physicians in temperate regions." This is, he continues, "a belief that is very erroneous and harmful."

This is a mild expression of a serious problem. The majority of physicians know little of amebiasis and what knowledge they have is usually incorrect. The disease is a national as well as a personal problem and one which is apparently becoming more serious. Yet the belief is still current that cyst-carriers are not a menace to themselves or others, just as the belief is still current that asymptomatic amebiasis actually exists and may safely be disregarded. The explanation of these erroneous concepts seems to be the general ignorance of the profession, for which in one sense they can scarcely be blamed: The whole subject of tropical disease is treated in a cavalier manner in most medical schools, if, indeed, any courses at all are given on the subject.

On the basis of our present knowledge, amebiasis seems a public health problem of serious magnitude, one which is worthy of the earnest consideration of health officers as well as clinical practitioners. What, then, is the solution? It is not, as McCoy and Chesley (13) emphasized, the examination of food-handlers, essential as hygienic precautions by that group may be. It is not the isolation of infected patients, according to the same authorities. That is neither necessary nor desirable, though it is essential, in their opinion, that in every instance the source of the individual infection be traced, a precaution that I fear is rarely observed.

The solution is certainly not such articles as "Man versus the amoeba," written for a state medical journal by a lay person in a smartly superficial style (31). It is not such articles as "The subtle murderer," which appeared in *Hygeia* (32) and which opened with the sensational statement that "The amoeba has been getting away with murder for years." Nor is it such articles as "Intractable amebiasis—warning!" which appeared in the *Military Surgeon* as a case report (33).

Education of the lay public is desirable, but not along such lines as these. Moreover, we must be careful to avoid what Alvarez (34), writing soon after the Chicago epidemic, termed amebaphobia. A nationwide psychosis on this sub-

ject could easily develop, he warned, and it is interesting to note that the educational campaign on cancer seems just now to have come up against this danger.

For the present, I believe that our best hope lies in the Committee on Amebiasis which was established informally during the International Congress of Tropical Diseases and Malariology in Washington in May 1948, and was later confirmed by the officers and councilors of the American Society of Tropical Medicine in executive session. The agenda for the opening meeting of this committee include research problems, diagnostic and therapeutic investigations, and educational endeavors, the idea being that all activities be conducted along the same lines as the similar activities of the committees which worked so effectively in other fields, notably malaria, under the Office of Scientific Research and Development during World War II.

Our present means of diagnosis are cumbersome, time-consuming, and utterly impractical for mass testing. Our present therapeutic methods cannot possibly be called satisfactory. And, finally, we cannot hope to educate the public concerning a disease about which the medical profession itself knows so little, though, on the other hand, we cannot hope to obtain from the public financial support for the investigation of a disease about which it knows nothing.

Our first problem, then, is the education of the general profession, our second the education of the lay public. I hesitate to suggest that we of this Society add to the already enormous medical literature, but my study of the literature of amebiasis during the years 1932-1947 has convinced me beyond doubt that our first duty, as specialists in this field, is the preparation for state, local and general national journals—not specialty journals or other journals which do not reach the average physician—of informative articles pointing out, for the benefit of the general practitioner, the basic facts of amebiasis as we now know them.

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SUGGESTIONS FOR THE FUTURE¹

GEORGE CHEEVER SHATTUCK²

I accepted the Presidency of the Academy of Tropical Medicine with deep feelings of personal gratitude to my colleagues, and also in the belief that they intended, through me, to pay a compliment to Harvard University for the work in tropical medicine which has been conducted there for many years.

In his presidential address to the Academy last year, Dr. Strode advocated federation of the American Academy of Tropical Medicine, the American Society of Tropical Medicine, the National Malaria Society and certain other groups. He pointed out that the membership of each of these organizations is small, and that our potential influence could be greatly enhanced by federation. The idea of federation has received general approval, and steps in that direction are being taken. The bodies joining in federation need not lose their identity. Each should continue to have its function.

My principal concern tonight is to suggest an expanded program for the Academy. You have heard Dr. Faust's resumé of its accomplishments. Organization of the Foundation for Tropical Medicine was delayed by the death of Dr. McKinley, who had been the chief exponent of the idea. Plans for fund raising were further delayed by a severe financial depression and subsequently by a world war. Nevertheless, the persistent work of Dr. Mackie and others finally brought the Foundation into being and it has helped to support teaching and research in tropical medicine.

Most of you attended the Congresses of Tropical Medicine and Malaria last spring. They were an outstanding success. The Academy can well derive satisfaction for having taken the initiative by proposing that the Congresses should be held here, for enlisting the support of other groups, and for the important part played by some of its members in organizing the program of the Congresses.

If the Academy is to be as useful as possible in the future, I believe that it should adopt a continuing program, and that it should assume new functions. For example, the Academy might:

1. Have a Standing Committee to keep in touch with legislation related to tropical medicine in the United States of America or abroad, and to recommend appropriate action from time to time.

2. The Secretary's office might also establish a clearing house for information required by teachers and students of tropical medicine and allied subjects. Information should be readily available about:

- a. opportunities for study or research in the U. S. A. or abroad;
- b. sources of financial support for individuals or projects; and
- c. activities of the World Health Organization, Inter-American Sanitary Bu-

¹ Presidential Address, American Academy of Tropical Medicine, Annual Meeting, New Orleans, December 7, 1948.

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reau, Research being conducted by United States Public Health Service, the Army and the Navy and also that going on in academic institutions.

3. Most important of all, the Academy should participate actively in the scientific meetings of the affiliated societies.

To this end, I suggest:

- a. that half a day should be allotted to the Academy for a scientific program;
- b. that the program should consist of a symposium on some broad medical problem of the tropics;
- c. that the subject should be approached from the standpoint of public health;
- d. that the most eminent persons conversant with the subject whether living in the U. S. A. or abroad should be invited to participate; and,
- e. that the addresses and abstracts of the discussion should be published by the Academy.

The time is ripe for a program of this character because interest in health in the tropics was aroused during the Second World War and this interest should be maintained and strengthened. Moreover, it is evident that for years to come American tropical medicine must play a leading role in the prevention and control of tropical diseases throughout the world.

As a subject for a symposium, the Academy might accept Dr. Strode's challenge of last year to explore the possibilities of controlling the effects of climate in the tropics. There are unsolved problems here of major importance. Air conditioning, of course, is possible but its use to the best advantage in the tropics requires much more knowledge than we have today.

Still more vital problems exist in the field of nutrition. You all know that most of the indigenous races of the tropics are undernourished. It is significant that the standard load for a porter in Liberia weighs only thirty pounds, whereas a porter should be able to carry fifty pounds easily enough. They cannot do so in Liberia because they are underfed, or ill, or both. If the tropics are to become an increasingly important bread-basket for the world, the laborer must be protected from disease and he must have sufficient food.

Nutrition is a subject of the highest importance for tropical medicine. Clearly, today it is a world-wide problem as well. We are becoming more and more conscious of the meaning of population pressure against food supply, which was pointed out by Malthus in the last century. Increase of population is being balanced to some extent at present by increased food production and by improved distribution. Nevertheless, in many parts of the world people are living now at a low subsistence level, while in other countries, there is widespread famine. Can people in the future obtain sufficient food when war, as we all pray, shall have ceased and when preventable disease in the tropics and elsewhere shall have been controlled? Will not population pressure against food supply lead ultimately to mass starvation? For how long can increase of food production counter-balance increase of population? A respite is possible by better distribution, by application of improved methods of agriculture and by cultivation of new land, so long as new land still exists. But students of conservation have said

that millions of acres of once cultivable land in the United States alone have been rendered sterile by mining the soil, or have been destroyed by erosion.

If you look at the muddy waters of the Mississippi you will see the wealth of a continent on its journey to the ocean. If you contemplate a barren hillside, you may realize that the ax has felled the protecting trees, that fire has consumed the humus and that rain has washed away the soil.

Where the turf of the windswept prairie has been broken by the plow, sooner or later comes a season of drought. The result is a dustbowl. Much of the top-soil is blown away and the dust in the air causes lurid sunsets. But it is the top-soil, and almost exclusively the top-soil, which provides food for man and beast.

Osborne and other students of the subject have pointed to the vital importance of soil conservation, and Vogt in his book entitled "Road to Survival" has dramatized the danger. He told me recently of a profitable coffee plantation upon a hillside in Venezuela. The forest above the plantation has protected it by controlling the run-off of the rain, but now there are plans to clear the forest and to plant the land to corn. If this is done, the coffee plantation will soon be washed away. Will the higher land long continue to yield corn? Certainly not. It, too, will be washed down the mountainside and no productive land will remain there. This sort of destruction of the soil is going on nearly everywhere in the tropics with the result that potential food production is being continually reduced.

Can we as physicians, sanitarians and medical scientists help to solve these vital problems of nutrition? Perhaps we can be of some service in this field. I bespeak your earnest consideration of that question.

THE AMERICAN ACADEMY OF TROPICAL MEDICINE

A BRIEF SKETCH OF ITS FOUNDING, ITS PURPOSE AND ITS ACCOMPLISHMENTS

ERNEST CARROLL FAUST¹

Organization of the American Academy of Tropical Medicine was the natural culmination of a need experienced by American physicians and medical scientists to integrate clinical and research activities in tropical diseases, to sponsor education in this field, and to discover ways of financing these objectives. Following informal conferences, an organization was effected in Washington, D. C. in February, 1934, and was incorporated the same year as a non-profit corporation under the laws of the District of Columbia. The late Earl B. McKinley, then Dean of the Medical School of George Washington University, was the stimulating personality in the formation of the Academy. Mr. Perry Burgess, President of the Leonard Wood Foundation, obtained funds for defraying the expenses of organization, as well as for publication of the comprehensive volume entitled "A Geography of Disease", which appeared in 1935 under Doctor McKinley's editorship. As an indication that the founding group planned this as an American undertaking, Article I of the By-Laws of the Academy restricts active membership to citizens of the United States of America, although honorary members were not limited as to nationality.

The purposes of the Academy, as expressed in the Charter, were

- (1) "To further the extension of knowledge for the prevention of human and animal diseases of warm climates;"
- (2) "To provide a current survey of work in progress in tropical medicine;"
- (3) "To coordinate American work in tropical medicine;"
- (4) "To function as a central source of information;"
- (5) "To cooperate with other agencies interested in maintaining and obtaining support for tropical medicine;" and
- (6) "To receive funds and administer them through grants-in-aid and in support of definite projects to the purposes and aims of the Academy."

At the organization meeting Theobald Smith was elected President; Charles F. Craig, Vice-President; Earl B. McKinley, Secretary; W. W. Cort, Treasurer; and Stanhope Bayne-Jones, Herbert C. Clark, Richard P. Strong, Alfred C. Reed and Henry E. Meleney, Council members. It was planned to confine the charter membership to 50, including the officers and councilors.

The First Annual Meeting of the Academy was held in New York City in April, 1935. This meeting was actually planned for late 1934 but was unavoidably postponed. It was decided that subsequent annual meetings should be held in conjunction with those of the American Society of Tropical Medicine, and it was voted to accept an invitation to affiliate with the American Associa-

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tion for the Advancement of Science. Malcolm H. Soule has represented the Academy on the Association's Council since 1936.

The Second Annual Meeting, which occurred in November, 1935, featured a symposium on "Economic and Social Features of Tropical Medicine in the United States." At that session the first honorary members were elected, namely, Professor Emile Brumpt, Professor G. H. F. Nuttall and Doctor C. M. Wenyon. Ernest Carroll Faust succeeded Doctor McKinley as Secretary and has held that post to the present time. In 1936 Professor Wilhelm Schüffner, and in 1937 Sir. S. R. Christophers, Doctor H. da Rocha Lima and Professor Jerome Rodhain were elected honorary members. In the latter year Thomas T. Mackie became Treasurer, and worked tirelessly to finance the program of the Academy.

As soon as money became available, an Advisory Council was formed and a committee was appointed to consider requests for financial assistance. Subsequently this Council developed into the American Foundation for Tropical Medicine. George C. Shattuck has ably served as the Academy's representative on the Board of the Foundation. Doctor Mackie was its first Executive Director and more recently has been its efficient President.

Due to the gracious generosity of President Cloyd H. Marvin of the George Washington University, the Theobald Smith Medal became available for award by the Academy on alternate years to individuals who have made "outstanding contributions to tropical medicine." The first award was made in 1937 to Marshall A. Barber, the second in 1939 to Richard P. Strong, the third in 1941 to Edward R. Stitt, the fourth in 1943 to Charles F. Craig, the fifth in 1945 to Charles Morley Wenyon, and the sixth in 1947 to Clay G. Huff.

In 1940 the category of emeritus membership was established, honorary membership was then limited to distinguished foreign workers in the field, and active membership was increased from fifty to seventy-five.

With the incorporation of the American Foundation for Tropical Medicine under the laws of the State of New York, a medical advisory committee elected by the Foundation from the membership of the Academy, served in selecting Latin American fellows for special postgraduate training in tropical medicine in the United States, in approving grants-in-aid for research projects, in subsidizing teaching programs and in providing financial assistance to American publications devoted to tropical medicine.

In 1943 a committee was appointed by the President of the Academy to "investigate and make specific recommendations relative to the Academy's activities in relation to the American Foundation for Tropical Medicine." This committee, of which Wilbur A. Sawyer was Chairman and Ernest Carroll Faust Secretary, made recommendations (published in *Science*, **99**, April 21, 1944) which may be summarized as follows:

- (1) The main objective should be stimulation, expansion and improvement of facilities for graduate and undergraduate instruction in tropical medicine in the United States, and
- (2) The Foundation should assist or bring into being a strong school or

department of tropical diseases in the South, on the Atlantic Seaboard and on the Pacific Coast.

In December 1944 Mark F. Boyd pointed out that the time was ripe for holding the Fourth International Congresses on Tropical Medicine and Malaria and, in his 1945 Presidential Address, he proposed that the Academy take the initiative in requesting other interested organizations to join in a resolution, petitioning the U. S. Department of State to sanction and to extend an official invitation to the Interim organizations of the Congresses to meet in the United States. At the same session the American Society of Tropical Medicine, the National Malaria Society and the Southern Medical Association voted concurrence in this resolution, and by Spring, 1946 the Secretary of the Academy announced that several other societies indicated their desire to participate. Through untiring conferences on the part of Louis L. Williams with personnel of the Department of State, official sponsorship was obtained. In the Spring of 1947 Wilbur A. Sawyer became the Executive Secretary of the Organizing Committee of the Congresses, and following a year of intelligent planning and preparation the Congresses were held in Washington, D. C. during May 1948. The large, distinguished official and non-official delegations from nearly all countries of the world, the splendid scientific program and exhibits, the gracious hospitality, and the entire esprit de corps of the Congresses all demonstrated how well the undertaking had been conceived and planned. While members of many societies and governmental groups worked loyally to achieve the unqualified success of the Congresses, the initiative and much of the continuing stimulus must be credited to the Academy.

In June, 1947 the officers and council members, acting for the Academy, wired their congressmen requesting support of the Mundt Bill (H. R. 3343), authorizing continuation of the International Information and Cultural Affairs Program of the Department of State. In April, 1948 the Academy joined the American Society of Tropical Medicine in a formal letter to each member of the House Rules Committee, requesting action on the bill authorizing the United States to become a member of the World Health Organization.

In December, 1947 the Academy, the Society and the National Malaria Society met conjointly in Atlanta, separate from the Southern Medical Association. Born of necessity, since the Association could no longer provide hotel accommodations for guest organizations, the meetings demonstrated that tropical medicine had come of age and could stand on its own feet.

The writer of this brief biographical sketch has had intimate acquaintance with the Academy since the First Annual Meeting. Elected as a charter member by the Organizing group, he has participated in most of the Academy's activities, including its periods of depression and its achievements. He is certain that progress has been made along the lines proposed by the founding group and he is confident of the Academy's future usefulness.

THE TROPICS AND THE WHITE MAN

THE THIRTEENTH ANNUAL CHARLES FRANKLIN CRAIG LECTURE

HERBERT C. CLARK¹

From the time of the invasion of the tropics by the white race of the north the question of what happens to it when permanently established in the tropics has been studied and debated. It is still a controversial subject.

How can one study the race and its succeeding generations over a long period of time in regard to the effects of climate *per se* without being able to isolate the race from all social, economic and disease factors? Considerable evidence has accumulated from animal experimentation and from physiological investigations but the problem of colonization has not been completely solved.

Price (1) in his book *White Settlers of the Tropics* divides the white races into those of North European ancestry and those of South European ancestry. He is also careful to separate the *sojourner* from the *permanent settler* whose objective is colonization. One of his conclusions is as follows: "To the racial purist the future that lies before the white settler in most parts of the tropics is far from promising."

Mills (2) insists that migration from the temperate zone to the tropics does indeed entail a developmental retardation, no matter how great the precautions taken to avoid dietary deficiencies and disease dangers. He thinks that scientific evidence in recent years indicates that man responds to his climatic environment in no uncertain manner. His energy level and vitality, his inventiveness and ingenuity, his progressiveness and ability to accomplish things, all seem to depend largely on the degree of climatic stimulation to which he is subjected. Lack of storm changes and too high a mean temperature level lessen his energy and aggressiveness. On the other hand, Mills in discussing mental stability states that a prolonged sojourn in tropical calmness and warmth will often make possible desired mental relaxation, just as it reduces physical load or stress on the heart. Chronic arthritic disorders cause less discomfort when the individual resides in the tropics. Some believe that climate cannot be accurately reproduced experimentally even when temperature, humidity, air movement and dust content of the air are controlled.

Cilento (3) courageously states that the conquest of climate is essentially the conquest of disease.

Manson, Sambon, Guiterras and Gorgas (4) believed that climate *per se* has no deleterious effect on the white man. Gorgas (5) believed that any part of the tropics could be colonized by the northern white race if protected against disease and supplied with safe water, food and shelter.

Chamberlain (6) with experience in the Philippines and the Panama Canal, claims that by far the large part of sickness and death formerly attributed to tropical climate is not due to climate *per se* but to isolation, tedium, nostalgia,

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venereal diseases, alcoholic excess, and to infection with specific parasites whose invasion is now almost entirely preventable. He feels that facts justify the hope that the progress of tropical sanitation may ultimately permit the permanent colonization of certain parts of the tropics.

Castellani (7) does not believe it possible for the northern white race to colonize the low, hot, moist regions of the tropics.

Manson and Sambon (8) direct attention to the fact that in 1665 eight Dutch soldiers were sent by The Netherlands East India Company to the little island of Kissa, sixteen miles off the most easterly point of Timor. A fort was built but the Company forgot all about this lonely outpost and they soon realized they were marooned. They had their wives with them. They set to work to build houses and cultivate the land. The descendants of these couples still remain. In two and a half centuries the 16 have risen to 300 and they are a strong, sturdy race showing no signs or evil effects from interbreeding. They still keep their blood pure. They work hard and the consequence is that after 250 years in this tropical island they are still fertile, indeed prolific, and still keep their Northern European characteristics. These facts are taken from the *The Dutch East* by MacMillan Brown. Australia is slowly colonizing with Northern Whites the best part of their arid regions and thus far are pleased with the results. Observations on later generations are still very much to be desired.

There are many colonies of both Northern and Southern European whites in Brazil who have thrived for years and even a few generations under somewhat unfavorable climatic conditions. Others have failed or degenerated.

Dr. Vincent (9) always took pleasure in pointing out the fact that Malthus in 1798 attempted to forecast the future of the population of the globe. It is significant that he included the tropics in his purview. He disputed vigorously the idea then current that tropical food supplies were automatically abundant. Dr. Vincent believes that the trend of population and the source of future food supply must be toward the equator. He also feels that there is a temptation, perhaps unduly, to exult over what has already been accomplished in sanitation and that we should keep in mind the difficulty of climate, international relations and the intermingling of races. Given all of the benefits of modern civilization he feels there might still exist the hazard to a fullness of health in the low tropics.

Broek (10) takes up the question of pioneering in new lands. After 400 years of migration by the European peoples, there are still large areas which they have avoided or only thinly settled. The remaining sparsely settled regions are those in which climate is a serious drawback—the hot wet regions, the hot dry regions the cold regions. At least it is not considered today that these regions are uninhabitable; the problem is to find techniques for overcoming the climatic drawbacks; but this involves high costs. There is an increased interest in colonization at present because of the search for places where European refugees can settle. The view is becoming more optimistic about the ability of the white man to remain healthy in these regions. It seems quite possible with good diets, proper sanitation, regular exercise, good housing and perhaps air conditioning. All of these things imply a high standard of living. There will probably not be much settle-

ment in such regions by large numbers of the white races unless there are very powerful reasons for it. The fact remains that there are potential regions for immigration. The majority of the whites who at present live in the tropics as sojourners or settlers, belong to the social-economic upper strata relying on other races for the laboring class.

Johnson (11) recently discussed tropical deterioration or tropical neurasthenia as it occurred in white troops during World War II. Evidence was collected by medical surveys in Pacific and Asiatic areas where fighting was prolonged and severe. Troops were efficient after continuous presence for as much as 2 years in severe tropical environments. Men react, under certain well recognized types of stress, similarly in tropical, temperate and cold environments. Tropical deterioration in relation to nutritional requirements offered no support to the proponents of a large intake of vitamins or a low intake of animal protein.

Bazett (12) states that the primary need of the tropics is the assurance of an adequate food supply; the second is the provision of improved living conditions. He claims that emphasis has been placed on the disability of individuals to do muscular work in the tropics but that such work can be performed efficiently; it is limited only in quantity, not in quality. Mental work on the contrary suffers on both counts. He claims that the impression is unwarranted that large scale air-conditioning of indoor working and sleeping quarters in the tropics is impracticable. Such conditions could be attained in Bombay throughout the year with an energy load for air-conditioning which would be far below the normal heat load for heating houses in Philadelphia or New York through the winter.

World War II has increased the growing apprehension of the tremendous rise in world population and the fear that in another century the food supply will not be sufficient. The future is pointing more and more to the tropics and the world seems to believe that it is for the northern white man to decide the course of development in those regions.

William Vogt, Pan American Union conservation expert writing in the United States Department of Agriculture Publication "Foreign Agriculture" says the conservation problem in Latin America is even more serious than in the United States. He estimated that South America now has about 2.2 acres of land per capita suitable for growing food. He believes that by 1983, merely as a result of population increase, the amount of arable land will be decreased to 1.1 acres per capita. This adds another reason that will operate against any idea for colonization by the white race in the tropics of our hemisphere.

We have all had opportunities in recent years to see the invasion of the tropics by large American business organizations and to observe the building and operation of the Panama Canal. Such organizations have required only their executive and administrative personnel on tropical locations, sojourners rather than settlers. The remainder of the employees and labor forces have of necessity been drawn from citizens of the countries in which they operate. Our assistance should be directed to the *home, health and transportation problems* for the improvement of all races in the tropics. Small migrations into the tropics by the northern white race will in a few generations be lost because of race intermingling. It

is worth repeating here the statement of Price: "To the racial purist the future that lies before the white settler in most parts of the tropics is far from promising."

The economic integration of the world will eventually force a solution of the white man's place in the tropics.

The knowledge of the effects of climate upon the northern white man is so incomplete that no answer can be given at present except to cite the Dutch Colony on the island of Kissa, the white settlements in Australia, Brazil and the Canal Zone. The facts only will be secured from a study of many later generations of such settlers. We, at present, are forced to confine our observations to the sojourner of many years residence in the tropics. I fully realize that the monotony of the tropical climate is not as interesting as the changing seasons of the temperate zone, but in all countries there are mountains where people can, if they wish, seek a cool climate without the time and cost of a trip to the temperate zone. When I recall the summers that I have spent in Philadelphia, New York and Washington, I can truthfully say that I prefer Panama. There, at least, the second part of the night is cool. I have seen and heard of more cases of heat exhaustion and sun-stroke in the cities named than has been true of my long residence in the tropics.

Perhaps the only new bit of information that I can add to the subject of climate in the tropics is to give you my own personal history since 1909. It was never my intention to locate in the tropics. An unexpected arrangement was made for a temporary tour of duty of six months in the Panama Canal Zone. I had been a victim of an extensive generalized case of psoriasis from 1905 to 1909 and the best authorities in Philadelphia held little hope of a cure of this skin disease. I gave the disease no attention after my arrival in Panama but the lesions spontaneously disappeared within two months. The winters of 1917 and 1918 were spent in France and during the second winter the disease reappeared in a more or less general manner. On my return to Panama it again spontaneously disappeared in about two months.

While on duty in the United States for the fall and some of the winter months of 1938 the skin lesions reappeared in the scalp, over the shoulders and a few patches on the back. On my return to Panama they disappeared in the usual time. It never bothers me in the tropics. I can offer no reason for its disappearance unless it be the correct relationship of light, heat and moisture. Summer time in the United States never caused it to disappear. I have always been fortunate in having a fair part of my duty out of doors and this fact as well as my interest in hunting, fishing and bowling provided the needed exercise that, in my opinion, is even more important in the tropics than in the temperate zone.

My medical history, otherwise, since 1909 is as follows:

1911 appendectomy caused by an accident during a diving contest at Taboga Island. Something happened in the right side of the abdomen and I believed it to be some pulled muscle fibers. I was helped out of the water and taken to Ancon Hospital for observation. The next morning the surgeons reported that the appendix had apparently been coiled up and attached in a post cecal position

and the accident had torn it away from its attachment and gangrene of the entire length of the appendix had followed.

1912 was the next illness. Otitis externa, bilateral and severe.

1926 Bacillary dysentery while on temporary duty in Haiti.

1927 Dengue fever while on temporary duty in Haiti.

1933 Thyroidectomy for multiple adenomata-colloid goiter. I shall always believe this followed an accident in June 1917. We were hunting deer on horse-back and I was told the horse I rode was used to having riders fire from his back. I got a shot and the horse ran away. He ran under the low hanging branches of a big tree. My right leg was hooked over the saddle as I turned low and backward but just the same a limb of fair size struck me on the left and front of the neck. No marked swelling occurred at the time but my neck was black and blue for several days. Two months later, while engaged in bowling, something happened in the left side of my neck that felt like someone had stung me with a small rubber band. Before the evening was over a lump the size of a hen's egg had formed, probably a hemorrhage. It completely disappeared during the next two years but in 1925 the collar band of my shirt had to be changed from a number 15 to a 16 and by the fall of 1933 I wore a number 17 collar band and Dr. Crile decided on operative relief. It may be pure coincidence but I feel that the accident of 1917 helped prepare the way for the cystic goiter.

1934 Tonsils removed.

1941 Dengue fever in Panama. Ever since 1933 I have made it a practice to examine my urine every two months expecting sometime to find a cast or two and perhaps a trace of albumen but nothing was found until the attack of dengue in 1941 when a trace of albumen was found and to my great surprise the sugar test was positive. Only at long intervals does this test prove positive but the blood sugar has remained on the average at 150. Never at any time have any parasites been found in my alimentary tract or in my blood films.

Over the years I have been host to 16 *Dermatobia hominis* larvae scattered here and there over the body.

Since 1933 I have set aside two or three days for a *health audit* in whatever medical center of the United States happened to be convenient for me. Perhaps some who are interested in climatology and physiology would like some of the reports made by these clinics.

Blood Pressure records from 1933 to 1947 made in four different medical centers ranged from 145 over 80 to 168 over 88. The average has been about 150 over 90.

Electrocardiograms were made in 1941 and 1947. Sinus arrhythmia was reported the first year and a normal report in 1947.

Eye refractions have been made from 1920 to 1948. Reading glasses were first used in 1920 and bifocals since 1942.

Basal metabolism tests were made seven times. In 1933 prior to the removal of the left lobe, isthmus and part of the right lobe of the thyroid gland it was -8 and -6 . In 1934 it was -3 . From that date to 1947 it ranged from -15 to $+30$. No thyroid extract has been taken since 1935.

X-Ray examinations of the chest and abdomen were done at three health audits and all were reported negative.

Blood cell counts were done in 1933, 1941 and 1948. All were normal for both the red and white cells.

Differential counts were done in 1933, 1941 and 1947. These were all reported as normal.

Hemoglobin estimations were done in the same years and they varied from 94 per cent to 104 per cent. Many tests made in Panama and the Canal Zone have never revealed an estimation below 90 per cent.

Blood chemistry examinations were made in 1933, 1941 and 1947. Prior to the removal of the thyroid in 1933 glucose was reported as 113 mg. per 100 cc. of blood. In 1941 it was 145 and in 1947 it was 161. Eighteen times in the Canal Zone the reports ranged from 145 to 239 but the average has remained at about 150. In only 6 tests made frequently in Panama did the urine show a positive test for sugar. Renal function tests made five times were within normal limits. Albumen and casts have never been found in the urine except during the second attack of dengue fever.

Non-protein nitrogen tests have been made 18 times with a range of 32.7 to 46.9, an average of 35.1 mg. per 100 cc. of blood. Sedimentation rate ranged from 12 to 30 in sixteen tests. The average was 20.

These health audits were made in Cleveland, Philadelphia, Baltimore, Canal Zone and Panama.

I have lived and worked in the Central American tropics for 37 years, and 34 years in the temperate zone. The tropics have been very good to me. However, I have been able to live a protected life in sanitated areas with proper food and water supply, and my duty has seldom required hours after sundown in an unsafe region. Nevertheless, I find it hard to believe that the time will ever come when it will be necessary for the northern white race in vast numbers to colonize the tropics. We know from the results of years of experience by large American business organizations in the tropics that the white sojourner can do very well over long periods of time if prepared and willing to live under proper discipline in regard to quarters, food, water and general sanitation. Perhaps climate, per se, as it affects man has been over emphasized. It is my belief that the northern white sojourner with the assistance of native tropical labor and artisans will produce and transport the necessary food products and other necessities without colonization of the tropics by that race.

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THE OCCURRENCE OF ENDAMOEBA POLECKI, PROWAZEK 1912, IN MACACA MULATTA AND IN MAN¹

JOHN F. KESSEL² AND H. G. JOHNSTONE³

INTRODUCTION

A study of the intestinal protozoa from several species of *Macaca* available for examination in Peking, China, by Kessel, (1928_a) and of domestic pigs from the same area by Kessel, (1928_b) showed that both monkeys and pigs harbor natural infections of intestinal protozoa which appear to be identical with the intestinal protozoa of man.

In addition, the pigs were found to harbor another amoeba, *Endamoeba polecki* Prowazek, 1912, which differed from *E. histolytica* in that the cysts were mononucleate in character and it did not grow successfully in the medium of Boeck and Drbohlav as did *E. histolytica*. In 1928 Kessel did not report *E. polecki* in the monkeys examined.

Recently in Los Angeles a study was begun in which several amebicidal agents were to be tested against the natural infections of *E. histolytica* in *Macaca mulatta*. See Kessel and Kaplan (1949). In a series of forty-two monkeys selected because most of them harbored *E. histolytica*, twenty-six were also found to harbor an amoeba which appears to be similar to *E. polecki* described from the pig (Kessel, 1928_b). Salis (1941) reported the presence of an amoeba of monkeys which produces uninucleate cysts. For this he accepts the name of *E. chattoni* Swellengrebel, 1914.

When the current observation was made the results were reported to Dr. H. H. Anderson and Dr. Herbert G. Johnstone of the School of Medicine, University of California, San Francisco, who were studying monkeys in a closely related problem. Slides were sent to them and they confirmed the observed differences and also sent exchange slides from their animals showing amoebic cysts of identical morphology. Dr. Johnstone has also encountered from two human cases, cysts of amoebae which varied in morphologic appearance from the cysts of *E. histolytica* and which reminded him of the cysts of *E. polecki*. We have both checked the slides from these cases and incline to the view that in morphologic appearance the amoebae of human source are the same as *E. polecki*. Prowazek (1912) also reported this amoeba from a child in Saipan.

DESCRIPTION OF ENDAMOEBA POLECKI

The descriptions of *E. polecki* by V. Prowazek in 1912 are insufficient to determine exactly the morphology of the amoeba he named, especially since he describes

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a binucleate cyst under the term "copulation cyste". However, subsequent workers who have discussed this amoeba, Wenyon (1926) and Kessel (1928) have accepted Prowazek's name and consider other proposed names for similar amoebae of the pig. e.g. *Endamoeba suis*, Hartmann (1913) and *Endamoeba deblickei* Nieschulz (1923) as synonyms of *E. polecki*. The descriptions and figures of Swellengrebel (1914) and of Salis (1941) for *Endamoeba chattoni* are so similar to those of *E. polecki* that one cannot but raise the question as to whether these two amoebae are identical morphologically. The host-parasite relationships and the question of whether physiological differences exist will have to be determined before this question can be finally answered.

Trophozoites of *E. polecki* from the monkey and the pig observed in fresh fecal preparations possess characteristics which relate them more closely to *Endamoeba coli* than to *Endamoeba histolytica*. The movement is sluggish as a rule, rather than active, and the pseudopodia do not possess the hyaline-blade like formation characteristic of actively moving trophozoites of *E. histolytica*. The trophozoites of *E. polecki* are coarsely vacuolated (Pl. 1, Fig. 1), and usually contain ingested bacteria, yeasts and other protozoa. No apparent differences were noted between the trophozoites as observed in monkeys and pigs. Motile forms of *E. polecki* were not observed in fresh or stained preparations in the cases reported from man.

Although frequent attempts were made to cultivate *E. polecki* from the monkey and from the pig on R.E.S. and liver infusion serum media, the results have not been successful. However cultures were obtained with stool material collected from one of the human cases. A washed fecal suspension containing numerous cysts was inoculated into tubes of a medium consisting of a coagulated emulsion of egg with Ringer's solution, an overlay of Locke's solution and rice starch. For the initial inoculation and first four transplants penicillin was added to the cultures in the proportion of 500 units for each cubic centimeter of overlay solution.

Microscopic examination of material removed from the cultures demonstrated actively motile amoebae, some of which contained starch grains and bacteria. In contrast to the trophozoites of *E. polecki* observed in preparations from the monkey and pig, the culture forms of the human case possessed rounded hyaline pseudopodia and demonstrated active progressive motility. Such characteristics are usually associated with *E. histolytica* and not with *E. coli*. It must be borne in mind however that valid distinctions cannot be made unless conditions at the time of observations are comparable. It is of little practical diagnostic value to make comparisons between trophozoites of amoebae occurring in stools with those growing in culture. The type of movement and the method of formation as well as the appearance of pseudopodia are characteristics frequently utilized as differential criteria. These however are so influenced by environmental factors that unless conditions are parallel they should not be employed.

The cysts are usually spherical and contain a single nucleus (Pl. I, Fig. 2-11). Only rarely has a binucleate cyst been observed. The diameter of cysts ob-

served to date has ranged from 8 microns to 15 microns, the average being about 12 microns. The chromatoidal bodies are rod-shaped or spherical. The rods possess angular or rounded ends, the angular ends at times being pointed. The chromatin material on the nuclear membrane is evenly encrusted or arranged in beads or plaques. The karyosome is usually massed and large, occasionally being small, Fig. 2 and at times dispersed, Fig. 5-9.

A structure frequently noted in the cysts in this study is a dark staining mass, spherical, ovoidal or somewhat irregular in shape which will be designated as the inclusion mass. It does not appear to be glycogen because it does not stain like glycogen with iodine and does not leave a clear space in cysts stained with iron-hematoxylin. In slides stained with iron-hematoxylin this mass appears at times like a thickening of the cytoplasm, but in iodine-eosin stains, the mass stands out quite distinctly as a separate structure. This was figured by Kessel 1928 (Pl. I, Fig. 16) although no comment was made concerning the same. A similar structure is figured by Salis 1941 (his figures 12-14 and 19). Salis, however, designated these structures as glycogen. In the material reported in the current study, this inclusion mass has frequently been found in cysts from monkeys, from man and from the pig. In Plate I, all figures except 6 and 11 show this inclusion mass.

It is not possible at present to do more than speculate upon the nature of this inclusion mass, but since it is present in some cysts and not in others this material could be considered as stored food substance which may be used as the cyst ages.

DISCUSSION

The frequent occurrence of natural infections of *E. histolytica* in monkeys, e.g. Castellani (1908), Dobell (1928), Kessel (1928_a), Hegner (1930), and Johnson (1941), has suggested the possibility that animals so infected might be used advantageously as test animals for amebicidal agents. Indeed, Anderson and Koch (1931) and Bond, Bostick, Hansen and Anderson (1946) have reported such studies. The findings of certain of these workers in which invasion of *E. histolytica* into the intestine was observed prompted this laboratory to attempt a study with available *Macaca mulatta*. *E. histolytica* was encountered in the preliminary examinations as anticipated. In addition, mononucleate cysts of an amoeba were found which were indistinguishable from the cysts of *Endamoeba polecki* Prowazek 1912 as reported from the domestic pig. This same observation was made by Salis 1941, who applied the name *Endamoeba chattoni* Swellengrebel 1914 to the amoeba. The occurrence of an amoeba in monkeys, the cysts of which might be confused with the mononucleate cysts of *E. histolytica*, unless critical differences are noted, is important to recognize, especially, since monkeys which harbor natural infections of *E. histolytica* are frequently used in studies on experimental amebiasis either in comparative pathology or in evaluating the effect of amebicides. Kessel and Kaplan (1949) show that the effect of certain arsenicals tested in monkeys is different against *E. histolytica* and against *E. polecki*, the same being less effective in eradicating *E. polecki*.

Since it is also suggested in this report that *E. polecki* may occasionally occur in man, where it probably lives as a commensal just as it does in the pig and in the monkey, it is also of value to differentiate *E. polecki* from *E. histolytica*. The writers recognize the possibility that the names *E. polecki* and *E. chattoni* represent different species based on physiological differences. They do not believe that host location alone is sufficient to warrant assigning a different species name, and for the time being they use the name *E. polecki* Prowazek 1912 pending any recognition of host-parasite relationship differences that may exist.

SUMMARY

During a study of the occurrence and treatment of natural infections of *E. histolytica* in *Macaca mulatta*, another amoeba was encountered which produces only mononucleate cysts, but which possesses rod-shaped chromatoidal bodies and nuclear structures which may be similar to those of *E. histolytica*. Judged on the basis of morphology this amoeba appears to be identical with *E. polecki* Prowazek 1912 of the domestic pig and with *E. chattoni* Swellengrebel 1914 of monkeys. An amoeba with similar morphological appearances has also been found in man.

E. polecki often possesses a spherical or ovoidal inclusion mass in the encysted stage and this structure differentiates it from cysts of *E. histolytica* or of *E. coli*.

E. polecki appears to be a commensal in the domestic pig and in the monkey. When found in the monkey it does not respond to amebicidal therapy as readily as does *E. histolytica*.

The similarity of these amoebae as here reported is based only on morphological structure since there has been no opportunity to attempt reciprocal animal infection experiments.

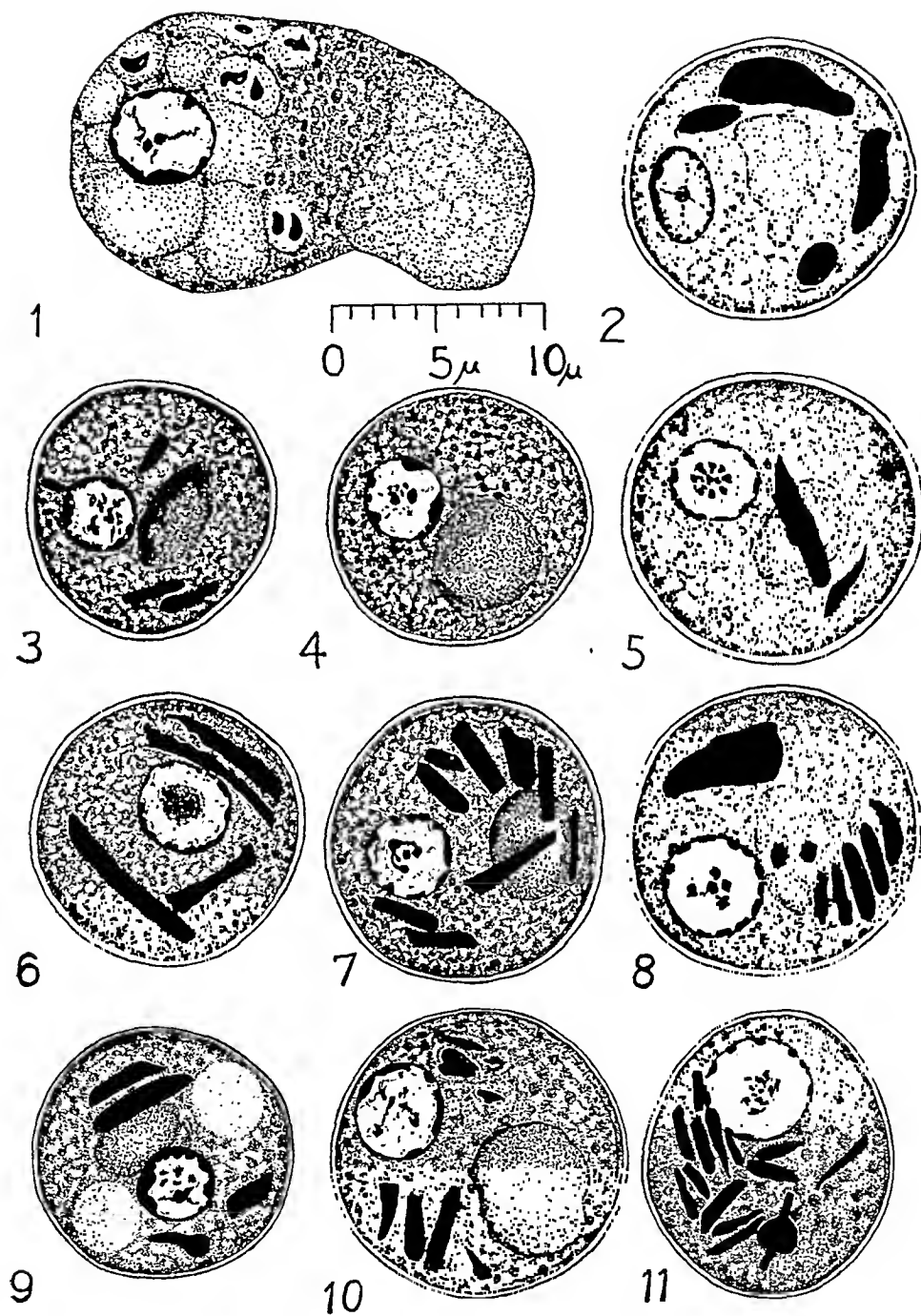
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1. Trophozoite of *E. polecki* from monkey. Note coarse vacuoles.
- 2-6. Cysts of *E. polecki* from monkeys. All show presence of inclusion mass except No. 6. Chromatoidal bodies are rod-shaped, some with rounded ends but most with angular ends. No. 4 shows cysts without chromatoidal bodies. Karyosome varies from massed, central type to excentric and dispersed type.
7. Cyst of *E. polecki* from pig. Note inclusion mass, characteristic chromatoidal bodies and karyosome in division phase.
- 8-11. Cysts of *E. polecki* from man. All show rounded to oval inclusion mass except No. 11. No. 9 shows presence of two glycogen vacuoles in addition to the inclusion mass. All show characteristic chromatoidal bodies.

PLATE I



THE EFFECT OF CERTAIN ARSENICALS ON NATURAL INFECTIONS OF *ENDAMOEBIA HISTOLYTICA* AND OF *ENDAMOEBIA POLECKI* IN *MACACA MULATTA*¹

JOHN F. KESSEL AND FAY KAPLAN²

INTRODUCTION

A number of workers have pointed out the occurrence of natural infections of *Endamoeba histolytica* in monkeys, and since chronic amebiasis together with tissue invasion is known at times to occur it has become common practice to use monkeys which harbor such infections as test animals for amebicidal agents (See Bond, Bostick, Hansen, and Anderson, 1946).

Salis (1941) and Kessel and Johnstone (1949) report that in addition to *E. histolytica* and other intestinal protozoa similar to those common in man, a certain number of monkeys harbored a species of amoeba which produces only mononucleate cysts and that these cysts might easily be confused with mononucleate cysts of *E. histolytica*. Salis applied the name *Endamoeba chattoni* Swellengrebel 1914 to this amoeba but Kessel and Johnstone point out that it appears to be identical with *Endamoeba polecki*, Prowazek 1912, which was first found in the domestic pig. Accurate differentiation between *Endamoeba histolytica* and this amoeba is necessary if one is to evaluate the true effect of amebicidal agents, since *E. polecki* probably is not a tissue invader and lives only in the lumen of the intestine. The purpose of this report is to compare the effects of certain amebicidal drugs on natural infections of these two amebae in monkeys.

PROCEDURES

Macaca mulatta recently imported from India were available in this laboratory for examination. Six stools from each monkey, collected on nearly consecutive days were examined in an attempt to select monkeys which harbored *E. histolytica*. Preliminary examinations were made using saline-iodine-eosine preparations. Iron-hematoxylin slides were made for a permanent record from all animals found to be positive for *E. histolytica* by the preliminary examination. Because of the similarity of the cysts of *E. polecki* and the mononucleate cysts of *E. histolytica*, in the preliminary stain, differentiation was not made at first by this method, and it was done only when the permanent slides stained with Heidenhain's iron-hematoxylin were examined.

Animals found to be positive for *E. histolytica* were placed in individual isolation cages and kept in the same during the period of the experiment. The period of drug administration lasted ten days, the designed amount of the drug being administered directly to each animal by a small rubber catheter leading to

¹ Aided by a grant from Parke, Davis and Company.

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the stomach. A Luer syringe was attached to the catheter making administration of accurate dosage possible.

TABLE I
Melarsen oxide

All monkeys received a dose of 50 mg./kg. per day over a period of ten days.

MONKEY	<i>E. histolytica</i>		<i>E. polecki</i>		<i>E. coli</i>		<i>E. nana</i>		<i>Iodamoeba</i>		<i>Chilomastix</i>	
	BT*	AT†	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
4285	+	+	+	—	+	+	+	—	+	+		
4311	+	—	+	—	+	—	+	+	+	+	+	+
4330	+	—	+	—	+	+	+	+	+	+		
4332	+	—	+	—	+	+	+	—	+	—	+	+
4479	+	—	+	+	+	—	+	—	+	—		
4559	+	—	+	—	+	+	+	+	+	+		
4581	+	—			+	+			+	—	+	+
4602	+	—	+	+			+	+	+	—		

Conclusions: Seven of the eight animals harboring *E. histolytica* were negative in follow-up examinations. Five of the seven, harboring *E. polecki* were negative in the follow-up examinations.

* BT—Before treatment.

† AT—After treatment.

TABLE II
Mapharsen

All monkeys received a dose of 10 mg./kg. per day over a period of ten days.

MONKEY	<i>E. histolytica</i>		<i>E. polecki</i>		<i>E. coli</i>		<i>E. nana</i>		<i>Iodamoeba</i>		<i>Chilomastix</i>	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
4281	+	—			+	+	+	+	+	—	+	+
4436	+	—			+	—	+	+	+	—	+	+
4483	+	—	+	—	+	+	+	—	+	+		
4558	+	—			+	—					+	—
4579	+	—			+	—	+	—	+	—		
4716			+	—	+	—	+	—	+	—		
4470	+	+	+	+	+	—			+	+		
4479	+	—			+	+					+	+
4495	+	—			+	—	+	—	+	—	+	—
4688	+	—	+	+	+	+	+	—			+	+
4555	+	+	+	+	+	+	+	—	+	+	+	+
4560	+	—	+	+	+	+	+	+	+	+	+	+
4590	+	—	+	—			+	+	+	—	+	+
4689	+	—			+	+	+	—	+	—	+	+

Conclusions: Eleven of the thirteen animals harboring *E. histolytica* were negative in follow-up examinations.

Three of seven animals harboring *E. polecki* were negative in the follow-up examinations.

The follow-up examination to determine the immediate results of treatment were begun ten days after completion of medication, examination again being made of six stools collected at daily intervals.

RESULTS

Several arsenicals were selected for use in the treatment experiments, including Melarsen (oxide)³, Mapharsen³ and carbarsone. The summaries of the studies

TABLE III

Carbarsone

All monkeys received a dose of 7.5 mg./kg. per day over a period of ten days.

MONKEY	<i>E. histolytica</i>		<i>E. polecki</i>		<i>E. coli</i>		<i>E. nana</i>		<i>Iodamoeba</i>		<i>Chilomastix</i>	
	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT	BT	AT
4714	+	-	+	+	+	+	+	+	+	+	+	+
4715			+	-	+	+	+	-	+	-	+	+
4721			+	+	+	+	+	-	+	+	+	+
4744	+	-	+	+			+	+	+	+	+	-
4746			+	-	+	-	+	-	+	-	+	-
4753	+	-			+	+			+	+	+	+
4754	+	-	+	+	+	+	+	-	+	+	+	+
4757	+	-	+	+	+	-	+	-	+	+	+	+
4765	+	-	+	+	+	+	+	-	+	+	+	+
4560	+	-	+	-	+	-	+	+			+	+

Conclusions: All of the seven treated animals were negative for *E. histolytica* in follow-up examinations.

Three of the nine animals harboring *E. polecki* were negative in the follow-up examinations.

TABLE IV

Table showing effect of three arsenicals on *E. histolytica* and *E. polecki*

	<i>E. histolytica</i>		<i>E. polecki</i>	
	No. + B.T.	No. + A.T.	No. + B.T.	No. + A.T.
<i>Melarsen Oxide</i> 50 mg. per kg. for ten days	9	1	7	2
<i>Mapharsen</i> 10 mg. per kg. for 10 days	13	2	7	4
<i>Carbarsone</i> 7.5 mg. per kg. for 10 days	7	0	9	6

B.T.—Before treatment.

A.T.—After treatment.

are included in Tables I, II, III, and IV. The initial natural infections of intestinal protozoa for each monkey are shown in the column designated B.T. and the results of the follow-up examination after treatment are shown in the column termed A.T.

³ Melarsen (oxide) and Mapharsen are registered trademarks of Parke, Davis and Co., for oxophenarsine and oxophenarsine triazine, respectively.

In general, the arsenicals, in the dosages used in this experiment, had little effect on the intestinal protozoa other than *E. histolytica*. Infections with *E. histolytica*, however, were appreciably reduced. These results are in keeping with the conclusions of previous investigators who have shown that most commensal protozoa of the intestine are not affected by the usual amebicidal agents.

Since *E. polecki* has never been found to invade the intestinal tissue either of domestic pigs or monkeys, this amoeba may therefore be classed as a commensal. The arsenicals used in this study had slight effect on *E. polecki*, this being similar to their effect on the other commensal protozoa. The one exception was melarsen oxide which drug reduced to a certain degree both *Iodamoeba* and *E. polecki*.

Since the cysts of *E. polecki* and the mononucleate cysts of *E. histolytica* may be easily confused because of the similarity of their size, nuclear structure and their chromatoidal bodies, it is important that their differences be learned and noted if monkeys which display natural infections of these amebae are to be used in studies on drug therapy.

SUMMARY

A comparative study of the effect of three arsenicals against natural infections of *E. histolytica* in *Macaca mulatta* show that all three are effective in the doses used when administered daily for ten days.

The same drugs in the same dosages were much less effective against *E. polecki* in the same animals.

These differences point out the necessity of differentiating between natural infections of *E. histolytica* and of *E. polecki* in monkeys when amebicides are to be evaluated by testing them against natural infections of *E. histolytica* in monkeys.

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CHEMOTHERAPY OF EXPERIMENTAL ENDAMOEBA HISTOLYTICA INFECTION IN DOGS

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INTRODUCTION

The preliminary in vivo evaluation of potential antiamebic drugs has involved primarily the use of infections in kittens, monkeys or rats. While these infections have been useful, each has been associated with certain disadvantages. Although dogs were first observed to be susceptible to *Endamoeba histolytica* over 50 years ago, an exploration of the suitability of experimental infections in these animals for chemotherapeutic research has been neglected. The results of the present work indicate that under suitable conditions canine amebiasis provides a convenient and rigid test for the in vivo testing of amebicidal drugs.¹

MATERIALS, METHODS AND GENERAL PROCEDURES

The experimental animals included 29 male and 2 female dogs of various breeds procured from an animal pound. Healthy animals between approximately 6 and 12 months of age (as judged by an experienced custodian) and free of diarrhea or dysentery were selected. Before inoculation with *Endamoeba histolytica* all animals were determined to be free of amebae by direct microscopic and cultural tests and they were kept in individual cages under conditions which minimized the likelihood of acquiring accidental infections in the laboratory. Anthelmintic treatment with tetrachlorethylene and prophylactic rabies vaccination were routinely employed before the dogs were used for experimental studies.

The dogs were maintained throughout the observation period on a predominantly fish diet, supplemented during the latter part of the study with brewer's yeast. Canned salmon or mackerel, cat-food preparations consisting of 100% fish or 85% fish and 15% cereal, and dog biscuits (Friskies) were used in various combinations to supply a diet composed of from 50 to 100% fish.

All of the amebic infections were produced with the BN1H strain of *Endamoeba histolytica* and its associated bacterial flora.² The inocula consisted of known numbers of amebae from stock cultures grown in Locke-eggslant-Locke overlay medium (Reardon and Ress, 1939) and suspended in 2.0–2.5% mucin (Jones 1946) or of amebae taken directly from an experimentally infected dog. In addition to amebae, stock cultures contained *Streptococcus fecalis*, *Escherichia coli* and *Proteus* sp. (apparently *P. ammoniae*).

¹ We are indebted to Dr. A. C. Bratton, Jr. for encouragement and valuable guidance in the chemotherapeutic aspects of this study, to Dr. C. K. Banks and Mr. D. F. Walker, Jr. for supplying drugs, and to Miss Faith Ludwig for assistance in bacteriologic aspects of the work.

² We are indebted to Dr. Ashton C. Cuckler of the Merck Institute for supplying this strain of amebae which was obtained from a patient by members of the National Institute of Health, Bethesda, Maryland.

Inoculations were performed intracecally through the anus by means of a 10 or 20 ml. glass syringe equipped with thin walled, flexible, plastic (Tygon) tubing. The tubing was gently inserted a sufficient distance to permit inoculation in the vicinity of the ileo-cecal valve. Fecal samples for evaluating amebic infection were taken by colonic irrigation with 8–10 ml. of physiological saline, using the same apparatus employed for inoculations. Samples were taken from the distal third of the colon and permitted to settle before microscopic examination. These are slight modifications of the method of Faust (1930, 1931).

Evaluation of amebic infections included direct microscopic examinations following supravital staining (Beemer, 1947) and cultural tests. Cultures were made by inoculating specimens directly from the sampling tube into liver and egg-yolk infusion medium (Balamuth, 1946). Rice starch was added and the culture results were determined after incubation at 37.5°C. for 1–3 days.

Drugs employed in chemotherapeutic studies included emetine HCl, chiniofon, carbarsone, diiodo-oxyquinoline (Diodoquin), chloroquine (Aralen) amodiaquin (Camoquin³), sulfadiazine, succinylsulfathiazole, streptomycin and crystalline sodium penicillin G.

RESULTS

I. Observations on Host-Parasite Relationships Essential to the Evaluation of Canine Amebiasis for Chemotherapeutic Studies

Susceptibility, symptomatology and the course of untreated infections. Data from 31 dogs are available for analysis of susceptibility and miscellaneous aspects of early untreated infections. In addition, observations on untreated infections extending over periods ranging from 33 to 98 days in 8 animals permit a general description of established infections.

Seventeen dogs were inoculated with amebae from experimentally infected animals and 14 dogs received amebae from stock cultures. Comparison of the infectivity of amebae as related to their immediate source is not entirely satisfactory due to uncertainty regarding the number of trophozoites in samples obtained by colonic irrigation. However, either type of inoculum produced a high percentage of infections. In the animal-to-animal series of inoculations all of the 17 dogs developed an acute amebic colitis with an average prepatent period of 4.8 days and a range of 2–10 days (based on observations at 2–4 day intervals). In the culture-inoculated series 11 of 14 dogs became infected and an average prepatent period of 5.8 days (range 3–10 days) was observed. In this group the number of amebae inoculated was determined in each instance and the following results were obtained. Eleven dogs were injected with inocula containing from 0.7×10^6 to 3.4×10^6 amebae and 10 developed infections (the one failure in this group received 3.4×10^6 amebae). Three dogs inoculated with 7.0×10^3 to 1.8×10^5 amebae failed to develop patent infections during 19–28 days of observation, but these animals were not on a strict fish diet which subsequent experience has indicated is important. Infections successfully established by

³ Parke, Davis and Co. registered trade mark for 4-(7-chloro-4 quinolylamino)- α -diethylamino-o-cresol dihydrochloride dihydrate.

When experimental infections had been established in dogs maintained on a fish diet, the course of the infection for at least several weeks was sufficiently uniform to permit experimental studies. Certain aspects of the course of untreated infections are summarized in Figure 1. In spite of the lack of a quantitative procedure for evaluating the intensity of amebic colitis, it was evident that acute or subacute infections extend over at least 2-4 months with only slight fluctuations in degree of infection. In several instances negative findings in infected dogs have resulted from the examination of specimens taken from animals whose lower colon contained much fecal material. An additional factor probably contributing to variability in the findings on successive examinations was variation in the diets which ranged from 100% to 50% fish. An impression arising from numerous successive fecal examinations by the same operator and from

[illegible]

clinical observation of the animals suggested that following establishment of an infection the animals rapidly developed an acute and occasionally fulminating infection.⁴ The acute phase typically extended over 2-3 weeks and was followed by a subacute stage of indeterminate duration. During the acute phase defecation was painful and explosive with bloody feces distributed around the sides of the cage. Hemorrhage into the intestinal tract was so severe that hemoglobin levels in animals with long standing infection frequently fell to 50% of the normal value. During the subacute phase temporary abatement of the infection occasionally occurred as is illustrated by dog 71 (Fig. 1) which was negative for amebae between the 85th and 91st days after inoculation, after which amebae again became demonstrable.

⁴ Three animals not represented in figure 1 died after inoculation on the 5th, 19th and 29th days, respectively. However death may have resulted from concomitant causes.

formed to the descriptions in acute infections by Faust (1932) and by Faust and Kagy (1934). Lesions were most numerous in the distal third of the colon, but frequently the entire large intestine exhibited shallow ulceration with numerous bleeding points. Lesions were usually numerous in the region of the ileo-cecal valve and in at least 2 animals they occurred also in the terminal portions of the ileum.

Microscopic examinations of specimens taken by colonic irrigation from infected animals typically revealed numerous amebae, erythrocytes, epithelial cells and leucocytes. The amebae conformed to the classical description of living amebae from cases of acute dysentery in man with comparable phagocytosis of erythrocytes, motility and morphology. Characteristic histolytica morphology was observed also in fecal smears stained with iron hematoxylin. Active motility commonly persisted for several hours in specimens kept at room temperature and not excessively diluted with saline.

An exception to the usual resemblance to amebae from man occurred on one occasion in all of 12 dogs under observation. This alteration in motility and morphology was temporary and was associated with an increase in the canned salmon content of the diet. (Salmon and dog biscuits had been given on alternating days for 25 days but on 3 of the 4 days before the observation of altered motility and morphology only salmon had been given.) These amebae exhibited an *E. muris* or free-living ameba type of motility without differentiation between the endoplasm and ectoplasm. The organisms moved in a rather circular path without explosive pseudopod formation. Most of them were sub-ovate. Phagocytosed erythrocytes could not be detected and they contained instead numerous large globules resembling a lipoid or volutin-like material. However, these changes were not observed in the cultured specimens, which contained only amebae of typical histolytica morphology. Following stabilization of the salmon and dog biscuit diet schedule, the amebae regained a characteristic histolytica appearance. The significance of these changes is unknown.

The relationship of diet to amebic infections. Critical evaluation of the effects of diet has not been completed in this study, but experience thus far has agreed well with the findings of Faust, Scott and Schwartzwelder (1934) as to the utility of a fish diet in achieving and maintaining acute infections. Moreover, it has become apparent that deviation from a predominantly or strictly fish diet for a sustained period interfered seriously with the standardization of the infections. Evidence of vitamin B-complex deficiency occurred in 4 dogs maintained almost exclusively on canned salmon for 3-4 months. In these animals the entire oral mucosa developed a grayish-black appearance. The gums were eroded and the tongue developed an abnormally smooth appearance. Two of the animals developed tremors and one became partially paralyzed. The latter dog was unable to stand and appeared to be in terminal stages with violent convulsions. Administration of parenteral vitamin B-complex promptly relieved the acute symptoms and subsequent inclusion of approximately 400 mg. of brewer's yeast in the diet each day rapidly restored the animals to a healthy state. The nature of these symptoms and their response to treatment indicated that black-tongue

in varying degrees of severity had developed due to prolonged maintenance on canned salmon.

While the mechanism by which a fish diet accentuates amebic colitis in dogs is not understood, it is unlikely that a B-complex deficiency is involved. In the first place, the effects of a fish diet are manifested before a deficiency has had time to develop and, secondly, administration of B-complex to dog 69 which had a subacute infection was followed by an exacerbation of the infection. On the contrary, the requirements by *E. histolytica* in vitro for B-complex (Hansen and Anderson, 1948) and the experiences with dog 69 suggest that a deficiency may militate against the infection. This type of relationship between the nutritional state of the host and the course of a parasitic infection has been observed by Seeler and Ott (1944 and 1946) in avian malarial infections.

Observations on the immunology of experimental canine amebiasis. Data obtained from prolonged studies on untreated or uncured infections and from reinoculation results in animals cured of their initial amebic infections (see section on therapeutic studies) indicated in a general way the role of immunity to amebiasis. Untreated animals on a fish diet developed an increased resistance to *E. histolytica* which was manifested by the development of subacute infections. Following cure of their initial infections 4 of 5 dogs have been reinfected. However, in each instance reinfection was accomplished only after 2-3 reinoculations, the first of which were given 30-45 days after eradication of the initial infection. Since these reinoculation observations were made on a group of dogs in which animal-to-animal transfer of the amebae was used, the number of organisms in each inoculum was not determined, but in all instances the inoculum was of a size that regularly produced infections in dogs that had not been infected previously. The one animal that could not be reinfected was inoculated with large numbers of infective amebae on 4 occasions over a period of 4 months. This animal following recovery from its initial infection had become completely refractory to *E. histolytica* in spite of maintenance on a fish diet.

The reliability of technics used in detecting the presence of amebic infection. Examinations for amebae usually included direct smear and cultural tests. Review of the results of these examinations in all infected and untreated animals revealed the reliability of the methods for detecting amebic infection. In direct microscopic examination of colonic irrigation material, 228 tests revealed amebae in 183 instances—80% efficiency. In cultural examinations (using liver, egg yolk infusion medium plus rice starch, see Balamuth, 1946) of the same material, 169 cultures revealed amebae in 79 instances—47% efficiency. Positive direct examinations with negative cultures occurred in 59 instances and negative direct with positive culture results occurred only 8 times. The combined efficiency of direct and cultural examinations was approximately 85% as compared with 80% for direct examinations alone. Routinely, cultures were incubated for either 24 or 72 hours before examining for amebae depending upon the day of the week when samples were taken for culture. From a tabulation of the culture results with respect to these incubation periods it was evident that whether cultures were incubated for 24 or for 72 hours did not significantly affect the efficiency

of cultural methods. The efficiency of Balamuth's medium in culturing amebae from infected dogs was considerably less than that reported by Snorf, Foltz and Howard (1947) in studies on human amebiasis.

II. Chemotherapeutic Studies

After observing that experimental infections were sufficiently uniform to permit an evaluation of the effects of therapy, a series of experiments were conducted with several established antiamebic drugs. The chief purpose of these studies was to determine the degree of correlation between the chemotherapeutic response of canine and human infections rather than a comparison of one established amebicide with another or the determination of exact minimal effective dose of an established drug. For this purpose emetine HCl, chiniofon, carbarsone and diiodo-oxyquinoline were selected. A 10-day treatment period was arbitrarily chosen and the drugs were tested at 3 total dose levels—usually, at one-fourth, the equivalent, and 4 times the approximate total human dosages as given in the N.N.R. (adjusted to the weight of the animals). The daily dose represented one-tenth of each multiple of the total human dose. Further details regarding the treatment schedules are included in the results for individual drugs.

Emetine HCl. Although emetine had amebicidal activity in the dog, there was little, if any, difference between curative and fatally toxic doses. Infection, treatment and diet data are summarized in Figure 2. Six dogs served as untreated controls and 8 were treated with emetine. Two untreated animals (60 and 66) died during the experiment. Post-mortem examination on these animals indicated that amebiasis was the most probable cause of death in dog 60, while the death of dog 66 was mainly due to pneumonia. Emetine was given by intramuscular injection in doses of 0.25–4.00 mg./kg./day for 10 days or until the animals succumbed to the drug. The treatment schedules are indicated by lines drawn under the symbols indicating the results of direct and cultural tests for amebae. Doses of 0.25 mg./kg./day were tolerated but failed to exert therapeutic effect (dog 55). Doses of 1.0–4.0 mg./kg./day for 10 days were not tolerated but antiamebic action occurred. This was indicated by a diminution in the number of amebae in dogs 73 and 63 during treatment with doses of 1.0 mg./kg./day.

Dogs infected with *E. histolytica* and maintained on a fish diet were highly sensitive to emetine. The fatally toxic reactions in dogs 51, 63 and 73 indicated that dogs cannot tolerate the drug as well as man since these animals died following the administration of the equivalent of established total human dosages. Symptoms observed during intoxication included vomiting, loss of appetite, listlessness, accumulation of a frothy exudate in the mouth and defecation of much whole blood. Autopsies performed on dogs 62, 63 and 73 revealed marked gastro-intestinal irritation. The stomach and intestines were congested and contained large amounts of whole blood. Apparently massive hemorrhage into the gastro-intestinal tract constituted an important aspect of the toxic effects of emetine, in spite of the fact that it was administered intramuscularly. An evaluation of myocardial damage resulting from the drug was not attempted.

A review of the literature revealed that emetine produced clinical improvement in 7 of 9 dogs naturally infected with an ameba indistinguishable from *E. histolytica* (Ware, 1916). These observations were made under conditions which scarcely permitted evaluation of the curative action of the drug. Treatment consisted of intramuscular injection of emetine in total doses of 65 mg. to small dogs or 130 mg. to large or medium-sized dogs over a period of 2 to 3 days. Evidently the amounts administered were near the maximum tolerated level since two of the animals exhibited evidence of intoxication, which was fatal in one

DOG NO.	DAYS INFECTED BEFORE EXPERIMENT	EMETINE MG./KG./DAY	INFECTION	TREATMENT	DIET DATA	ON DAYS EXP	EXP. NO.
			+	+	+	+	
55	39	0.25	+	+	+	+	1
51	60	1.00	+	+	+	+	1
63	53	1.00	+	+	+	+	2
73	23	1.00	+	+	+	+	2
62	3	2.00	+	+	+	+	2
64	33	2.00	+	+	+	+	2
54	4	4.00	+	+	+	+	1
65	3	4.00	+	+	+	+	1
52	13	UNTREATED CONTROL	+	+	+	+	1
66	4	"	+	+	+	+	1
60	13	"	+	+	+	+	1
68	31	"	+	+	+	+	2
70	34	"	+	+	+	+	2
71	34	"	+	+	+	+	2

* DIRECT/CULTURE EXAM RESULTS, + AMEBAE PRESENT
- ABSENT

FIG. 2. PROTOCOLS OF EXPERIMENTS TO EVALUATE THE CURATIVE EFFECTS OF INTRAMUSCULAR EMETINE HCl AGAINST CANINE AMEBIASIS

Duration of treatment is indicated by lines extending across the days of observation and diet data are indicated as in fig. 1

instance. Post-mortem examination on this dog revealed marked inflammation of the gastric mucosa. Bauche and Motais (1920) and Boyd (1931) observed that dogs are inordinately susceptible to emetine and that gastro-intestinal irritation constitutes a prominent feature of intoxication along with myocardial damage. These authors failed to achieve cure of canine amebiasis with emetine HCl by parenteral administration or with emetine-bismuth-iodide by oral administration, although Boyd (1931) reported clinical response with emetine HCl.

Carbarsone. The amebicidal effect of carbarsone was readily demonstrated. Three dogs were treated by oral administration of the drug in gelatin capsules. Treatment consisted of 2, 8 or 32 mg./kg./day, respectively, administered b.i.d.

for 10 days (Fig. 3). Doses of 2 and 8 mg./kg./day were ineffective (dogs 63 and 64) but 32 mg./kg./day rapidly arrested the infection in dog 62. The sudden disappearance of amebae and erythrocytes from the stools during therapy and the absence of evidence of infection during a 37-day follow-up indicated that the infection had been eradicated. Obviously these data are inadequate for a comparative evaluation of carbarsone but this observation of curative action against acute amebic dysentery in the dog (although at a high dose level) served the primary purpose of the study, namely, to indicate qualitative similarity between the chemotherapeutic response of canine and human amebiasis.

Chiniofon. This drug cured in doses equivalent to that used in man and at

DOG NO.	DAYS INFECTED BEFORE EXPERIMENT	TREATMENT		INFECTION, TREATMENT AND DIET DATA ON DAYS OF EXPERIMENT												EXP. NO.
		DRUG	MG/KG/DAY	5	10	15	20	25	30	35	40	45				
63	6	CARBARSONE	2.0	+	+	+	+	+	+	+	+	+	+	+	1	
64	6	"	6.0	+	+	+	+	+	+	+	+	+	+	+	1	
62	6	"	32.0	+	+	=	=	=	=	=	=	=	=	=	1	
67	4	CHINIOFON	4.0	+	+	+	+	+	+	+	+	+	+	+	2	
68	4	"	16.0	+	+	=	=	=	=	=	=	=	=	=	2	
65	6	"	64.0	+	+	=	=	=	=	=	=	=	=	=	2	
69	34	DIODO-OXYQUINOLINE	15.7	+	+	+	+	+	+	+	+	+	+	+	2	
72	40	"	63.0	+	+	+	+	+	+	+	+	+	+	+	2	
75	46	"	225.0	+	+	+	+	+	+	+	+	+	+	+	2	
62	13	UNTREATED CONTROL *		+	+	+	+	+	+	+	+	+	+	+	1	
66	4	"		+	+	+	+	+	+	+	+	+	+	+	1	
60	13	"		+	+	+	+	+	+	+	+	+	+	+	1	
68	20	"		+	+	+	+	+	+	+	+	+	+	+	2	
70	34	"		+	+	+	+	+	+	+	+	+	+	+	2	
71	34	"		+	+	+	+	+	+	+	+	+	+	+	2	

* DIRECT / CULTURE EXAMINATIONS, + AMEBAE PRESENT
= ABSENT

FIG. 3. THE EFFECTS OF TREATMENT WITH CARBARSONE, DIODO-OXYQUINOLINE AND CHINIOFON

Treatment schedules are indicated as in fig. 2 and diet data as in fig. 1

4 times the human dose level. Three dogs were treated, respectively, with 4, 16 and 64 mg./kg./day for 10 days (fig. 3). Methods of administration were the same as that described for carbarsone. Dog 67, treated with 4 mg./kg./day probably was not specifically cured by chiniofon as amebae were demonstrable for 13 days after completion of therapy. This animal subsequently lost its infection while maintained on a salmon and dog biscuit diet. Experience has shown that infections may become less severe in proportion to the degree of deviation from an all fish diet and this factor probably was partly responsible for disappearance of the infection, although delayed action of chiniofon therapy may have contributed to recovery. Inasmuch as dog 67 was the only animal

refractory to reinfection (see section on immunology) immunity probably exerted unusual influence on the course of its infection. The infections in dogs 68 and 65 responded rapidly to treatment. Amebae could not be demonstrated in the animals by direct or cultural tests during the 33-38 days of follow-up and apparently the infections were cured. Symptoms of infection disappeared immediately after therapy was started.

Diiodo-oxyquinoline. Evidence of antiamebic activity of diiodo-oxyquinoline was observed in 3 dogs treated with 15.7, 63.0 and 252.0 mg./kg./day for 10 days, respectively (fig. 3). The drug was administered orally in capsules for two 5-day periods separated by a 2-day rest period. Reasonable evidence of cure was observed in dog 55 (treated with 252 mg./kg./day). Some amebicidal activity was observed in dogs 52 and 69, although the former was not cured. Dog 69 recovered from its infection although its stools contained amebae 4 days after completion of therapy. This finding is inconsistent with the failure of diiodo-oxyquinoline to cure dog 52 which was treated with 4 times as much drug. However, over all comparison of the course of treated infections with that of untreated infections demonstrated the activity of this drug.

Antibacterial agents. An important consideration in evaluating the suitability of canine amebiasis for chemotherapeutic studies involves the etiology of the disease. The relative importance of amebae and of bacteria in causing amebic dysentery has received much attention in the literature. An opinion frequently expressed is to the effect that bacteria act as the primary pathogens while *E. histolytica* acts as a feeble pathogen or commensal. While it can hardly be maintained that the following data give conclusive proof of the primary importance of amebae in causing dysentery in dogs, they minimize the importance of bacteria in so far as the most useful antibacterial agents are able to indicate.

Penicillin G, administered intramuscularly as the sodium salt to one dog, was completely ineffective. Treatment consisted of 10,000 units/kg./day for 10 days given b.i.d. (in the early morning and late afternoon). This dog was subsequently treated with a combination of penicillin G, streptomycin and sulfadiazine but this therapy was also ineffective. The course of treatment extended over 10 days and included 10,000 units/kg./day of penicillin G, 100 mg./kg./day of streptomycin and 150 mg./kg./day of sulfadiazine. All drugs were given b.i.d., the antibiotics by intramuscular injection and sulfadiazine by oral administration in gelatin capsules. Finally an animal was treated with succinylsulfathiazole in doses of 300 mg./kg./day for 10 days (b.i.d., orally in gelatin capsules). This relatively poorly absorbed sulfonamide exerted no significant effect upon amebic colitis. These data indicate the suitability of canine infections in evaluating the amebicidal activity of new drugs apart from their possible antibacterial action.

Amodiaquin. Since amodiaquin was amebicidal in vitro at a dilution of 1:45,000 (24-hour test against the University of Chicago strain of *E. histolytica* with mixed bacterial flora in Balamuth's egg-yolk infusion medium, dilution expressed in terms of drug base), trial of this antimalarial drug against amebic colitis in the dog was indicated. Details of this work are summarized in Table 1. Although oral doses as low as 5 mg./kg./day exhibited antiamebic action in

causing a diminution in the number of amebae and an alleviation of symptoms, cures were not obtained at or near the maximum level tolerated either as a single large dose or by repeated administration over a period of 10 days. Definite evidence of drug damage to the parasites was obtained with doses of 10 mg./kg./day. The few indentifiable amebae during therapy usually resembled dead organisms. In addition to loss of motility, some had outstretched pseudopods while others were approximately round but contained large granular areas in the cytoplasm surrounded by a wide hyaline zone. Phagocytosis of erythrocytes was clearly inhibited. Most of the organisms stained with eosine which has not been observed with motile amebae during extensive use of supravital staining with an eosine-containing solution.

Chloroquine. This drug was found to be amebicidal in vitro at a dilution of 1:35,000 (expressed as drug base), using a test similar to that employed in

TABLE 1

Results of treating amebic colitis in dogs with amodiaquin and chloroquine

DOG NO.	DAYS INFECTED BEFORE THERAPY	TREATMENT			RESULTS
		Drug	Dose* mg./kg./day	Duration and schedule	
68	92	Amodiaquin	2.5	10 days b.i.d.	Not cured
87	11	Amodiaquin	5	10 days b.i.d.	Not cured
86	11	Amodiaquin	10	10 days b.i.d.	Not cured
68	113	Amodiaquin	50	Single dose	Not cured
84	45	Amodiaquin	100	Single dose	Died
89	18	Amodiaquin	200	Single dose	Died
88	11	Chloroquine	10	10 days b.i.d.	Not cured
86	33	Chloroquine	20	Single dose	Not cured
80	10	Chloroquine	40	Single dose	Died

* Expressed as free base.

determining the amebicidal activity of amodiaquin. This finding, in conjunction with the reports of Conan (1948 a and b) of considerable in vitro amebicidal activity and of apparent activity against human amebiasis, suggested trial of the drug against canine amebiasis. Three dogs were treated by oral administration of the drug in gelatin capsules (table 1). Significant therapeutic effect was not achieved in the 2 animals surviving treatment although the amounts administered were at or near the maximum tolerated levels for repeated or single dose schedules of therapy (Gruhitz 1948).

DISCUSSION

The work of Faust (1930, 1931, 1932) Tobie (1940) and their associates revealed that, contrary to the previously prevailing opinion, dogs are highly susceptible to *E. histolytica*. This contribution in conjunction with the discovery by Faust, Scott and Schwartzwelder (1934) that dogs develop and maintain an acute amebic colitis when kept on a fish diet has provided an almost ideal experimental infection for the evaluation of potential antiamebic drugs.

The present paper represents largely a practical application of these discoveries with particular emphasis on the exploration of experimentally-induced canine amebiasis for chemotherapeutic studies. The experimental data presented here indicate a high degree of uniformity in canine infections (established and maintained under optimal conditions) and good correlation between the response of canine and human infections to treatment. All of the four established antiamebic drugs tested were active against amebic colitis in the dog and, significantly, treatment with massive doses of representative antibacterial agents failed to modify the course of these infections. Dogs have been considered as cured when amebae could not be demonstrated during a 3-4 week period including two and usually three direct and cultural examinations per week. An additional criterion of cure was the absence of bloody stools after specific therapy. On this basis cures were attained with carbarsone, chiniofon and diiodo-oxyquinoline but not with emetine.

The techniques employed in the inoculation of dogs with amebae and in procuring specimens for following the course of the infection apparently do not contribute significantly to the pathologic processes associated with amebic colitis. Trauma is not involved in the inoculation process since a small flexible tube is gently inserted through the anus. Evidence that the sampling technique does not contribute significantly is afforded by study of the protocols (figs. 1, 2, 3) which record each examination made during the indicated observation periods. The data in Figure 1 illustrate clearly that dogs maintain severe infections when tubal specimens were taken at 1-3 week intervals.

It is highly desirable to evaluate potential antiamebic drugs against acute infections which can be provided best by keeping the animals on a fish diet. Obviously, the ideal drug for treatment of amebiasis is one that is effective against both acute and chronic infections. Failure to observe cyst formation in dogs on fish diets does not appear to be a serious disadvantage. The greater sensitivity of trophozoites as compared with cysts to various chemical agents indicates that chemotherapy should be directed against trophozoites which may or may not form cysts in man depending upon the severity of the infection.

Use of the dog in evaluating potential antiamebic drugs seems to avoid most of the disadvantages associated with the other hosts commonly used, namely, monkeys, kittens and rats. Rhesus monkeys are commonly infected in nature by an organism indistinguishable from *E. histolytica*. These infections are usually chronic and of uncertain duration which prevents their standardization and increases the difficulty of evaluating the effects of therapy. In addition, Kessel (1947) has found rhesus monkeys are infected with another ameba, *E. polecki*, which is easily confused with *E. histolytica*, and the response of these two organisms to chemotherapeutic agents is quite different. The reports of Dale and Dobell (1917) and of Clampit (1948) concerning chemotherapeutic experiments with *E. histolytica* infections in kittens point out the serious disadvantage of a high mortality among these animals due to septicemia and pneumonia. Young rats have been used for chemotherapeutic studies by Jones (1946) and colleagues, and by Goodwin, Hoare and Sharp (1948) and their associates. Use of these

infections affords certain advantages associated with the use of a small animal, but one obstacle has been encountered. Namely, it is difficult in this country to secure rats not already infected with amebae, probably *E. muris*. Sampling of rats from several widely separated sources has revealed natural infections in the colonies of each supplier and similar findings have been reported privately by other workers. This problem has been encountered also in England by Goodwin, Hoare and Sharp (1948) and by Fulton and Joyner (1948). The presence of natural amebic infections in rats increases the difficulty of using these animals for chemotherapeutic studies, particularly since there is uncertainty regarding the correlation in chemotherapeutic response between these natural infections and those due to *E. histolytica*.

Several disadvantages arise in the use of dogs for antiamebic screening. These include obvious problems associated with the size of the animal including housing, management, maintenance and quantity of drug sample required for therapeutic studies. Ware (1916), Faust (1931), Boyd (1931) and others have described apparent *E. histolytica* infections that had been acquired in nature. These reports indicate the necessity of examining dogs for natural infections before using them for experimental work. (Natural amebic infections have not been encountered in examining dogs used in this study.) Considerable selection has been necessary to procure dogs relatively free of other intestinal protozoa such as trichomonads, *Giardia* and coccidia, which may contribute to the symptoms of experimental amebic infections. The control of distemper has been a problem as most of the animals were less than a year old and probably were first exposed to infection in the pound from which they had been recently procured. This problem was partially solved by the practice of holding a surplus of animals in quarantine for 2-4 weeks before selecting those suitable for experimental work. However, this practice increased the incidence of intestinal protozoa as it was not possible to house dogs in individual cages during this period. Approximately a dozen dogs ill with symptoms of distemper have been treated successfully with a combination of anti-bronchisepticus serum (Parke, Davis & Co.) and distemper vaccine (Pitman-Moore and Co.).

In spite of these disadvantages, experimentally-induced amebic infections in dogs appear to be very useful for the preliminary evaluation of potential antiamebic drugs, specifically affording a test infection with the following characteristics: (1) caused by human strains of *E. histolytica* in a host that is readily available at relatively low cost and that is well suited for experimental work; (2) sufficient susceptibility and uniformity to permit evaluation of therapy; and (3) good agreement with human infections in response to chemotherapeutic agents.

SUMMARY AND CONCLUSIONS

Dogs maintained on a fish diet were highly susceptible to infection with a human strain of *Endamoeba histolytica*, obtained from stock cultures or from an experimentally infected animal. Infected animals fed a strict fish diet developed and maintained acute or subacute infections for several months. The course of these infections was sufficiently uniform and mortality among infected animals was

sufficiently low to permit chemotherapeutic studies. The infection was primarily an amebic colitis; extra-intestinal infections were not encountered.

Treatment of the infections with established antiamebic and antibacterial drugs revealed satisfactory correlation between the chemotherapeutic response of canine and human amebiasis. Emetine HCl, carbarsone, chiniofon, diiodo-oxyquinoline and amodiaquin exerted antiamebic action in dogs. Cures were obtained with carbarsone, chiniofon and diiodo-oxyquinoline but not with emetine or amodiaquin. Chloroquin, sodium penicillin G, streptomycin, sulfadiazine and succinylsulfathiazole were ineffective under the conditions of this study.

The failure of the antibacterial agents—penicillin, streptomycin, sulfadiazine and succinylsulfathiazole—to affect the course of the infections indicated that amebae rather than bacteria are the primary etiologic agent in canine amebiasis.

Consideration of the relative merits of dogs, monkeys, kittens and rats for evaluating the activity of potential antiamebic drugs indicated that canine infections should be particularly useful in the search for new chemotherapeutic agents.

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ENDEMIC FULMINATING AMEBIC DYSENTERY

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Acute amebic dysentery has long been known as a disease of the tropics peculiarly liable to affect newcomers. A native population has always been considered to be immune from the acute phase of the disease. Manson-Bahr (4) in his "Dysenteric Disorders" states—"it is undoubtedly true that Europeans in the tropics as a rule suffer from amebic dysentery more frequently and severely than do native races." Craig (3) made similar observations about the Philippine Islands. Native populations generally have a very high carrier rate and it is considered that the immunity from acute symptomatology is due to long association with the parasite.

Here, in Durban, we have a climate in which the conditions can be described as tropical in Summer. Here also are three different races living together. First, the indigenous Bantu, who, in this area, are practically all Zulus and whose ancestors came to this part of the world sometime in the last 500 years. Secondly we have the European whose tenure of this area has been only the last 100 years. Thirdly we have the Indians who were brought over here as laborers about 50 years ago. These indentured Indians were, at the termination of their contract, given the opportunity of returning to India or remaining in this country and practically all elected to stay. The local Indian population now consists mainly of their descendants though there have been from time to time further importations from India and the East as ordinary immigrants. In this town, the Europeans live in well built houses or apartments adequately supplied with the essential services of water and sewage disposal. At least 95 per cent of the European homes have Africans as domestic servants living in. The Indians in general live in shanty-type dwellings on market gardens and the like around the town. In the town they live in European type dwellings. In their natural state the Bantu live in the characteristic bee-hive type hut which have been so much featured in photographs of Africa. However, when these Africans seek employment in the towns as laborers and industrial workers their accommodation constitutes a big problem. Certain employers have erected large compounds which are adequate for the male members of the Bantu population but do not make any provision for family life. Apart from these and a few Municipally-erected native villages there is practically no decent housing available for the local Bantu and they therefore live in shacks built of the most varied of materials and entirely without any question of water supply or adequate sewage disposal. The municipalities have reected stand-pipes supplying water at somewhat lengthy intervals and the sewage is generally speaking disposed of into pit-latrines in an unsuitable soil.

Dietetically, the European lives on a similar diet to people in America. Indians live on their classical diet of curry and rice supplemented by green vegetables. The peri-urban Africans live on a very poor diet indeed, maize meal

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constitutes the main item and the African manages to get very little of the vegetables or meat and certainly not the milk which he gets in his home kraals. Now, amebiasis effects these three races in three different ways. In the European, dysentery of any major degree is extremely uncommon and the symptomatology is in general vague and indefinite. Secondary complications such as amebic liver abscess are practically unknown. In the Indian, once again dysentery as such is rare but amebic liver abscess is not infrequently seen. In the African, who forms the main subject of this thesis, the disease presents as an acute fulminating dysentery with numerous stools consisting of little else than pus, blood and mucus. The stool differs from that of a bacillary dysentery only in the presence of innumerable actively-motile hematophagous amebae. It is not uncommon to find fields in which there are anything between two and three hundred amebae, and the appearance of amebae attached to the edge of a piece of mucus has been likened to a row of sucking pigs. Clinically, too the disease is much more severe. It is generally speaking easy to see the ulcers by means of a sigmoidoscope, for in those cases which come to post mortem the ulcers are found from end to end of the large colon and not infrequently as high up as the ileum. The mortality rate is very high being about 10 per cent and the two commonest immediate causes of death being perforation and dehydration. Cases not infrequently present with an acute abdominal condition. Perforation is the commonest cause of death and is usually found to be multiple. Other complications such as liver abscess are very common indeed and we have for example seen several cases of perforation of liver abscess into the pericardium. To give some idea of the number of cases seen, I may state that during the year 1947 I saw over 1,400 cases of acute fulminating amebic dysentery. I have been able to use this enormous amount of material as test series for different forms of therapy, the results of which are being published elsewhere (2). This paper is more concerned with the epidemiological problem. Here, we have three races living together and intimately exposed one to the other and yet they get the same condition in different degrees of incidence and severity.

Now, what are the factors which may be giving rise to the difference?

1) Is the African peculiarly sensitive to infection by this parasite? Were this the case such a dysentery would be found in other parts of Africa, but it is not. The author has been in other parts of Africa and has never seen any condition to parallel the acute amebic dysentery of the Bantu as seen here in Durban and surrounding areas. Similarly too, observers from other parts of Africa are astounded at the clinical appearance of the patient and the microscopic appearance of the stools when they visit my laboratory, so that we can state that a racial susceptibility is not the explanation.

2) What is the part played by diet in determining this infection? As pointed out earlier in the paper the African is primarily a maize-eater, whereas the Indian eats his traditional curry and rice, and the local European has a full diet. It would seem likely that there is some dietetic deficiency which is determining the infection. However, some of the arguments applied in the question of racial susceptibility also apply here. In other parts of Africa the African diet is just as deficient, it may be that in the peri-urban areas where the Africans are no

longer able to exist on their natural diet there may be additional deficiencies, but in peri-urban areas other than in Natal amebiasis is not seen in the same degree as it is here in Durban, if at all. Though certain vitamin deficiencies are not uncommon they are not in any particular way associated with amebiasis. Amebic dysentery is in some way connected with a maize diet, as is exemplified by the paper by Alexander and Meleney (1) quoting their Tennessee experiments, similarly Winfield and Chin (5) in comparing North and South China where on the one hand there is a maize eating population and on the other a rice eating population stated that the degree of severity of amebic dysentery differed very markedly between the two.

3) Is the severe amebiasis seen in Durban due to the particularly bad conditions of hygiene in the peri-urban areas? There can be no doubt at all that this plays a large part in the dissemination of the infection, but it may be pointed out that conditions elsewhere in other parts of Africa are just as bad. However, it is extremely likely that the local streams are very highly contaminated with feces and when one sees the enormous numbers of amebae in infected cases one begins to wonder whether it is not possible that the water supplies may be so contaminated with active trophozoites that these, and not cysts, are transmitting the condition. There is a certain association between the rainfall and the incidence of new cases. As was mentioned earlier the incubation period is short, histories of under a week being common. One can generally expect an influx of new cases after the first rain following the Winter drought. This first rain means that the streams begin to run again, and are washing infective soil down to the lips of the next African who may choose to take his drinking water from that stream. In such an area, as might be expected, flies are common and though certain measures are taken against them, there is no doubt that they too constitute a possible means of infection. Also, Durban is famous throughout South Africa for its cockroaches. These too may act as a carrier, mechanical or otherwise, of the condition. Nevertheless in this same area we have Indians and Africans living cheek by jowl and what applies to the one almost equally applies to the other in the question of hygiene, so this too does not constitute the whole story. Furthermore if these unsatisfactory conditions of hygiene were very serious no doubt we would have outbreaks of other diseases of fecal origin such as typhoid, but we do not. Certainly the incidence of the condition in the better conducted Native townships is significantly smaller than in our worst areas but nevertheless cases do occur as they do even in the barracks in town.

SUMMARY

In Durban, South Africa, three different racial populations live together. In one of these, the Native African, there is a high incidence of an acute fulminating rapidly fatal amebic dysentery. This form of the disease is not seen in either the European nor Indian inhabitants of the area. The three races differ as regards diet and to a certain extent as regards the conditions of hygiene under which they live. Certain possible factors giving rise to this difference between various races are discussed.

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PAMAQUINE POISONING IN MAN, WITH A CLINICOPATHOLOGIC STUDY OF ONE CASE¹

AAGOT CHRISTIE LÖKEN² AND WEBB HAYMAKER

Since the introduction of pamaquine (plasmochin) in the treatment of malaria in 1926 by Mühlens (15), there have been numerous reports on its toxic effect. Le Heux and Wijngaarden (13) observed that tolerance to the drug varies in different species. In rabbits, for instance, the lethal oral dose was 225 mg. per kg. body weight; in dogs it was 20 mg., and in cats only 7.5 mg. Pamaquine poisoning was characterized in its early stages by slow respiration, dyspnea, and slow pulse rate, and in later stages by methemoglobinemia and cyanosis. The concentration of methemoglobin in the blood varied: in cats it was at its height approximately 16 hours after administration and gradually fell to zero in about 7 days. Urinary secretion of the drug was found to be very scant and the mode of destruction in the body uncertain. *In vitro* experiments with blood to which pamaquine was added disclosed that methemoglobin formed more readily in hemolyzed than in nonhemolyzed blood. Pamaquine in a concentration of 0.01 per cent produced methemoglobinemia and slight hemolysis; in concentration of 0.1 per cent or higher methemoglobin formation was found to precede hemolysis.

Tskimanauri (20) observed in dogs and rabbits that therapeutic doses of pamaquine caused temporary tachycardia, slight elevation of blood pressure, and increase in depth and rate of respiration. In animals given pamaquine after bilateral vagotomy a slight elevation of blood pressure and bradycardia occurred. In pamaquine-treated animals with the vagi intact there was tachycardia rather than bradycardia. Moreover, larger doses of pamaquine had no material effect on the cardiac rhythm. These observations were taken to mean that pamaquine acted directly on the vagal center. Goodman and Gilman (8) have stated that pamaquine is toxic to the heart, causing tachycardia, extrasystoles and other arrhythmias, but they did not disclose the source of their information. Heimann and Shapiro (9) found in man that pamaquine increased the amplitude of the various deflections in electrocardiograms, particularly the T wave.

There does not seem to be any generally accepted dosage of pamaquine. According to Goodman and Gilman (8), the dose of pamaquine from which adults obtain maximum benefit is 0.02 gm. 3 times a day. Cecil (3) advised giving pamaquine in conjunction with quinine, or following the administration of quinacrine, in biweekly doses of 0.02 gm. until gametocytes had disappeared. Strong (19) and Mackie, Hunter and Worth (14) recommended 0.01 gm. by mouth 3 times a day for 4 days. In the United States Army's "combined QAP treatment," quinine is given first, then quinacrine, and after a lapse of 2 days, 0.01 gm. of pamaquine 3 times a day for 5 days (Jarcho (11)).

As to toxic effects of pamaquine in man, Berliner and Butler (1) have observed

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that they rarely occur when daily doses of 0.03 gm. are given, but are evident in 50 per cent of those receiving 0.06 gm. daily, and in almost all those given 0.09 gm. per day. West and Henderson (21) noted symptoms of intoxication in 24 of 846 patients with malaria (2.85 per cent) who were treated with 0.01 gm. of plasmochin 3 times a day for 5 days, the treatment starting 2 days after an 8-day course of quinine-quinacrine therapy had been concluded. The symptoms which appeared after the administration of 0.06 to 0.15 gm. of pamaquine consisted of headache, dizziness, abdominal pains, nausea, vomiting, jaundice, and slight fever. In 2 cases psychosis developed, and in a third, coma. Methemoglobinemia did not occur, hemoglobinuria was commonly observed, while albuminuria and biliuria were constant features. West and Henderson felt that individual reaction to pamaquine was inconstant and unpredictable.

Patients receiving pamaquine have usually been given either quinine or quinacrine, or both, shortly before pamaquine therapy was started. The question has been raised as to whether under such circumstances delayed action of quinine or quinacrine may have been partly responsible when toxic symptoms develop during subsequent pamaquine therapy. Also there are indications that pamaquine intoxication is more severe when pamaquine and quinacrine are administered together. In this connection, Chopra and Abdul Wahed (5) reported the case of a patient who had had "malignant malaria" for 6 years, who was treated with chinoplasmin³ both therapeutically and prophylactically without developing symptoms of intoxication. On one occasion the patient was given "a tablet of atabrine and one of plasmochin," each 3 times a day for 5 days, after which marked cyanosis, epigastric pains, and dyspnea developed. The symptoms subsided after 4 or 5 days. The authors interpreted this as evidence of the toxic effect of the combined therapy. Recently it has been established experimentally by Chen and Geiling (4) that while quinacrine and quinine act independently and similarly, pamaquine and quinine are synergistic in their joint action, and quinacrine and pamaquine act independently but diversely.

Kennedy et al. (10) found that with either oral or intramuscular administration of pamaquine, the maximum level of the drug in the plasma was reached in 1 to 2 hours, and the concentration fell virtually to zero after 8 hours. If, on the other hand, quinacrine was given just prior to pamaquine therapy, the plasma levels of pamaquine were from 2 to 10 times higher and the rate of disappearance from the plasma far slower. A minimal elevation of pamaquine values in the plasma occurred after a 0.01 gm. dose of quinacrine, and was demonstrable for 6 weeks after a single dose of 0.5 gm. They concluded that quinacrine interferes with the destruction of pamaquine and in that way increases its concentration in the plasma.

Instances of death from pamaquine are few, and the reports are usually not detailed. Blackie (2) presented one case. The patient, an adult white man, had had blackwater fever on 2 previous occasions following quinine medication. During another attack he took 0.2 gm. of quinacrine and 0.01 gm. of pamaquine

³ This is a preparation in which each tablet contains 5.0 mg. of pamaquine and 0.16 gm. of quinine.

3 times in 24 hours. Shortly after the third dose of pamaquine he complained of respiratory distress, but the treatment was continued for another 24 hours, by which time he was gravely ill. Cyanosis was marked. He had received a total of 1.2 gm. of quinacrine and 0.06 gm. of pamaquine. Death occurred on the third day of treatment. At autopsy, the bladder contained dark highly acid urine, which on spectroscopic analysis showed methemoglobin; the only other pathologic condition observed was "acute hemorrhagic nephritis." Simeons (18), in treating 5600 cases of malaria with 2 injections of 0.3 gm. of quinacrine 24 hours apart, followed by one tablet of 0.02 gm. of pamaquine on the 3 subsequent days, reported 4 cases of severe intoxication. In all 4 there was hemoglobinuria; death occurred in 2, but post-mortem examination was not done.

Cordes (6) reported 2 fatal cases, only one of which was described in any detail. This patient had taken 0.04 gm. of pamaquine twice a day for 3 days (combined with 1 gm. of quinine daily), a total of 0.24 gm. of pamaquine. On the fourth day headache developed. On the fifth day he was jaundiced, pale, somnolent, and his hemoglobin was 30 per cent. Blood smears showed polychromatophilia and atypical normoblasts. Death occurred on the sixth day. Autopsy disclosed slight enlargement of the spleen and liver, and on microscopic examination early centrilobular necrosis of the liver was observed. The spleen showed the picture of malarial infection, and the kidneys were edematous. Examination of the brain revealed nothing of note.

De Langen and Lichtenstein (12), in their text-book on tropical medicine, mentioned that 4 fatal cases of pamaquine poisoning were on record. They remarked that the incidence of fatality was doubtless higher, for they themselves had known of 7 unreported cases.

Owing to the paucity of reported fatal cases, it would seem that the record of one which reached the Army Institute of Pathology in 1944 (AIP Accession 124392) might contribute to a better understanding of the toxic effects of this drug.

Case report. The patient, a 23-year-old white man, entered hospital on 22 August 1944 with symptoms of chronic otitis media and mastoiditis on the right side. He had had intermittent drainage from both ears since the age of 11. On admission the drainage was inconspicuous.

In February, 1944, while in New Guinea, the patient had acquired malaria. It had responded well to quinacrine therapy, which had been discontinued one month before he entered the hospital, *i.e.*, July, 1944.

On 3 September, chills and fever of 104° F. developed, but the next morning the temperature was 98°. Another attack occurred on 6 September, and still another on 16 September, and on this occasion, *Plasmodium vivax* was found in blood smears. Antimalarial therapy was begun, the patient being given 0.66 gm. of quinacrine daily from 16 to 19 September (a total of 2.5 gm.), and 0.66 gm. of quinine thrice on 21 September and once on 22 September (a total of 2.5 gm.).

Due to a misunderstanding, the patient received 3 doses of 0.4 gm. of pamaquine (altogether 1.2 gm.) on 22 September. By the next morning he was cya-

notic and apprehensive, complained of nausea and of pain in the abdomen, back, chest and jaws, and had two bouts of vomiting. There was slight tenderness of the right flank. A transfusion of 1000 cc. of glucose-saline solution was given intravenously, followed by 500 cc. of whole blood. (Thereafter he received glucose-saline daily.) On 24 September the cyanosis was more marked, and there was slight abdominal rigidity. He had difficulty in swallowing, complained of blurred vision, and was very restless. His urine was of port wine color. On 26 September he complained of numbness of the face, had difficulty in speaking, and he was sweating profusely. Cyanosis persisted and he complained of difficulty in breathing. Another transfusion of 500 cc. of blood was administered. He remained deeply cyanotic for the next two days with little change in his condition, but on 29 September stridor developed. The soft palate was found to be paralyzed and edematous. Toward evening his respiration became gasping, and death occurred at 3:00 P.M., 7 days after the overdosage of pamaquine. The records of temperature, pulse, respiration and blood pressure are given in Fig. 1.

Laboratory Examinations. See Table 1.

Autopsy. (15 minutes after death). *Gross Observations.* The body was that of an asthenic but well developed individual, measuring 71 inches in length and weighing 155 pounds. The entire skin had a yellowish, cyanotic hue. The sclera showed no trace of icterus.

Spectroscopic examination of blood taken from the heart disclosed approximately 4 per cent methemoglobin. Analysis of liver and kidney for quinacrine yielded 6 mg. per 1000 gm., and in the urine there were 5 mg. per 1000 cc. Tests for pamaquine in kidney, liver and urine were negative. No malarial parasites were found in blood smears.

Examination of the thoracic and abdominal viscera disclosed relatively little that was noteworthy. The pleural and pericardial cavities were of normal appearance and free from fluid, while the pelvic cavity contained about 20 cc. of yellowish fluid. The heart weighed 370 gm. and was of the usual appearance. The lungs were air-containing except for the lower lobes, which were edematous and displayed several wedge-shaped hemorrhagic areas. The liver weighed 1850 gm. Its capsule was smooth and on section had an appearance suggesting slight fatty metamorphosis. The spleen weighed 280 gm.; its capsule was tense and its pulp congested. Examination of the gastrointestinal tract disclosed scattered small hemorrhages in the gastric mucosa. The kidneys together weighed 460 gm. Their capsules stripped easily. On section the cortices were found to be wide and the striae straight, though accentuated by engorged vessels and glomeruli. The remainder of the genitourinary tract was normal. The vertebral marrow was dark red. There was nothing remarkable about the appearance of the pancreas, thyroid, parathyroids or lymph nodes.

The brain was normal in size, shape, and appearance. Section revealed no abnormalities.

Microscopic Examination. *Thoracic and Abdominal Viscera.* The heart muscle showed the usual cross striations. There was no fragmentation. The inter-

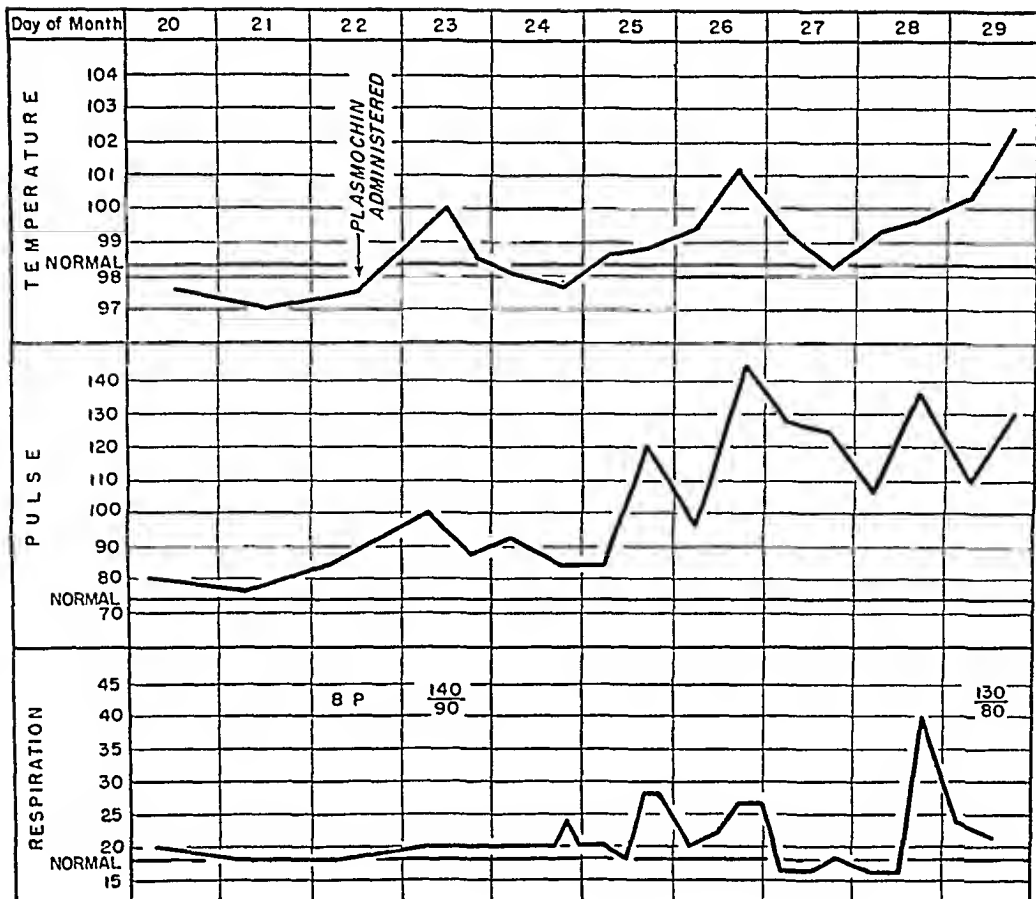


FIG. 1. TEMPERATURE, PULSE AND RESPIRATION CURVES
Blood pressure determinations are also indicated.

TABLE I
Blood and urine values

(+ signifies positive; O, negative; and —, no information available)

DAY (SEPT.)	BLOOD							URINE							
	Hemoglobin (per cent)	Red blood cells (millions)	White blood cells	Hemat- ocrit (per cent packed cells) (Normal: 45-47)	CO ₂ combin- ing power (vol. per cent) (Normal: 53-72)	Urea nitrogen (mg. per cent) (Normal: 10-15)	Ic- terus index (Normal: 4-8)	Specific gravity	Reac- tion	Albu- min	Blood		Sugar	Casts (Gran.)	Bile
											Chem.	Micro.			
23	90	4.5	23,000*	—	52.6	9.5	—	1.025	Alk.	+++	+	+	0	+	+
24	—	4.6	—	45	—	—	—	—	†	—	—	—	—	—	—
25	—	4.2	—	40	—	—	—	1.018	Alk.	+	+	+	—	—	+
26	90	4.4	—	—	—	—	—	—	—	—	0	—	—	—	+
28	90	4.5	9,550†	45	—	16.5	8	—	—	—	—	—	—	—	—

* Differential count (in per cent): Neutrophils 87, lymphocytes 10, monocytes 2, basophils 1.

† Neutrophils 50, lymphocytes 38, monocytes 9, eosinophils 2, basophils 1.

‡ Urine of port wine color.

stitial tissue was moderately edematous. The coronary arteries appeared normal. The lower lobes of the lungs were the seat of acute lobular pneumonia; there was also scattered moderate intra-alveolar hemorrhage. As to the liver, the lobular architecture was well preserved; there was slight to moderate centrilobular fatty metamorphosis and some malarial pigment in the Kupffer cells. In the spleen the malpighian bodies were of normal size and contained large germinal centers. The pulp showed nothing unusual aside from malarial pigment in cells lining the sinuses. Examination of the gastrointestinal tract disclosed only venous engorgement in the submucosa of the stomach. The kidneys exhibited uniform vascular engorgement. Epithelial cells of the convoluted tubules had indistinct borders and displayed cloudy swelling, while those of the distal nephron showed suggestive degeneration and some brownish pigment. No edema or leukocyte infiltrates were found in the interstitial tissues of the kidneys. The adrenals were within the range of normal. Nothing noteworthy was found in the pancreas, thyroid, larynx, or lymph nodes. The bone marrow showed active erythropoiesis.

Brain. The brain was fixed in 10 per cent formalin. Representative blocks were removed and embedded in paraffin and celloidin, and sections were stained by hematoxylin-eosin, cresyl violet, Bodian activated protargol, and by the Weil myelin method, the Holzer glia fiber method, and the Turnbull method for iron.

The most striking change was observed in the middle of the basis pontis. Here there was a focal degenerative lesion which measured approximately 4 mm. in cross-sectional diameter (Fig. 2A). Numerous nerve cells of the nuclei pontis in this area had disappeared and others were in various stages of degeneration. Myelin had disappeared and been replaced by rows of gitter cells; what remained of the nuclei pontis was also crowded with gitter cells (Fig. 2B). There were no apparent vascular changes and the Robin-Virchow spaces were free from extraneous cells. A search for thrombosed vessels was made, but none was found. There was no hemorrhage, nor were there hemosiderin-containing macrophages. The degenerative focus merged gradually with the surrounding normal parenchyma. A search for similar foci in the remainder of the brain stem was fruitless. The nerve cells in the nuclei pontis surrounding this area showed varying degrees of degeneration, generally mild.

Considerably less severe changes were observed in the globus pallidus. In the vicinity of vessels there were scattered nerve cells, microglia and macrophages loaded with brownish pigment, which when stained by Turnbull's method proved to be iron-containing. Cresyl violet stained sections disclosed scattered proliferation of microglia and astrocytes (Fig. 3A), an observation confirmed in Holzer-stained sections (Fig. 3B). The changes were found only in the internal division of the globus pallidus.

The cerebral cortex had also undergone changes, but of a relatively minor nature. The laminar pattern was not altered, but scattered nerve cells, particularly in lamina III, had hyperchromatic nuclei which sometimes were peripherally placed and swollen. The cytoplasm was sometimes vacuolated. The degenerated nerve cells appeared to have no special relation to blood vessels.

No alteration of glia was noted, except for a slight increase in oligodendroglial satellites. Blood vessels displayed no evidence of proliferation. No changes

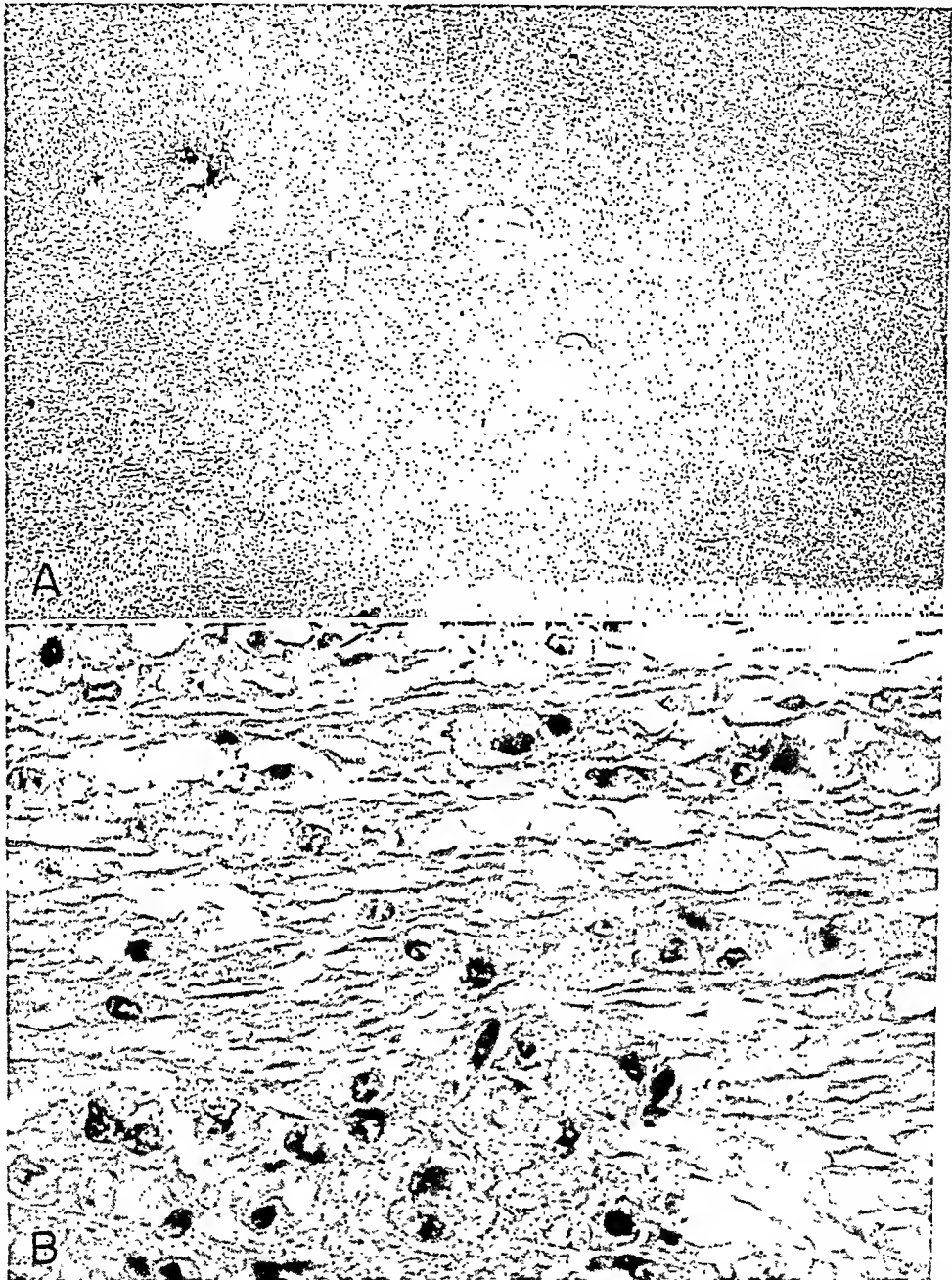


FIG. 2. A. Area of focal degeneration in the mid-region of the basis pontis. ($\times 22$)
B. Field from A, showing replacement of nuclei pontis and fibrae pontis by large macrophages. (Hematoxylin-eosin stain, $\times 500$.)

were observed in the hippocampal formation except near the hilus of the dentate gyrus where a group of cells of the pyramidal layer were acutely ischemic.

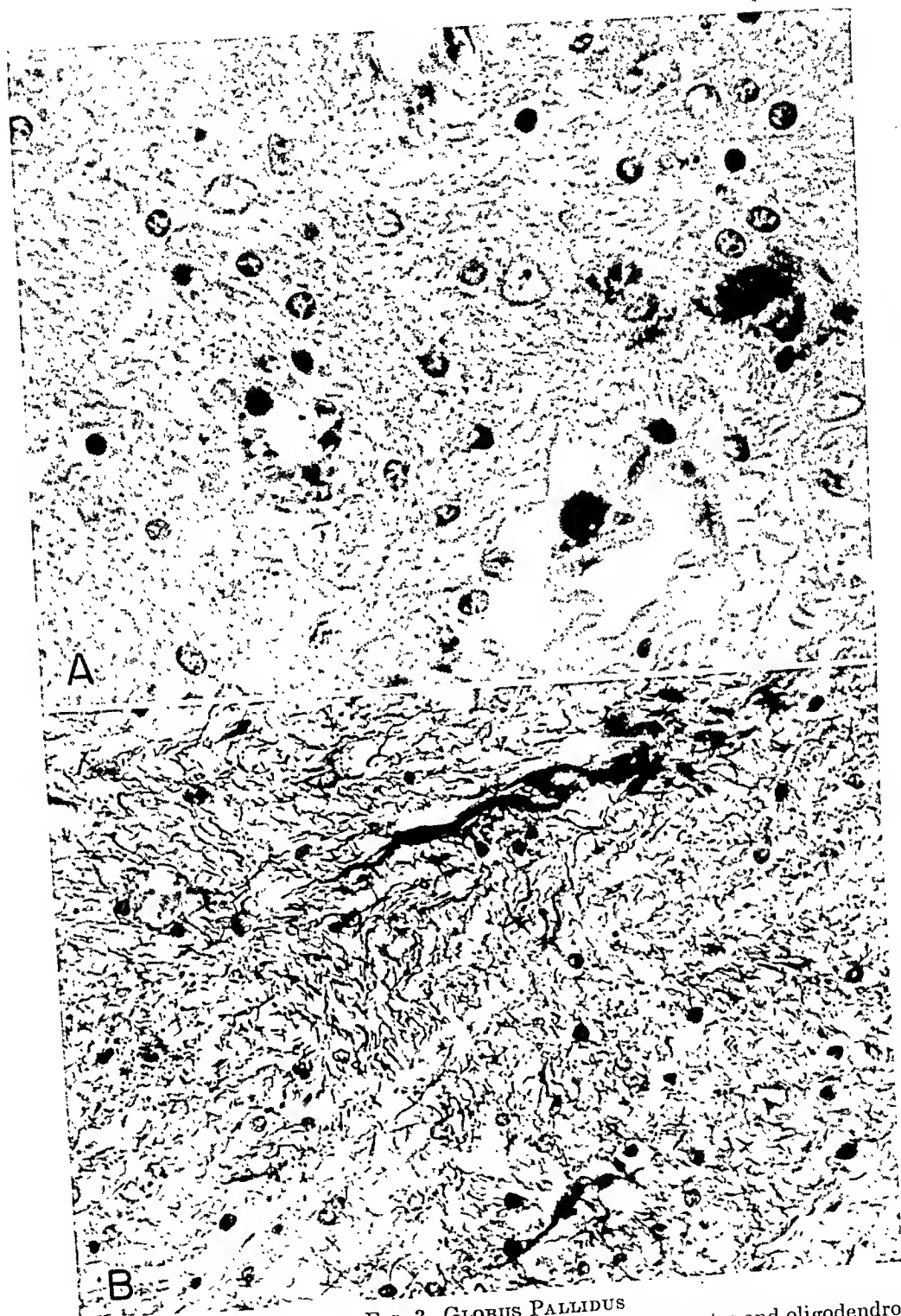


FIG. 3. GLOBUS PALLIDUS

A. Great reduction in ganglion cells and proliferation of astrocytes and oligodendroglia. Pigment is present in nerve cells and macrophages. (Turnbull stain showed the pigment to be iron-containing.) (Cresyl violet, $\times 705$.) B. The fibers of proliferated glia in the same area. Occasional cell bodies of astrocytes are also to be noted. (Holzer stain, $\times 400$.)

The leptomeninges displayed little that was noteworthy except over the convex surface of the brain where a few trabecular histiocytes had developed into free, large mononuclear cells.

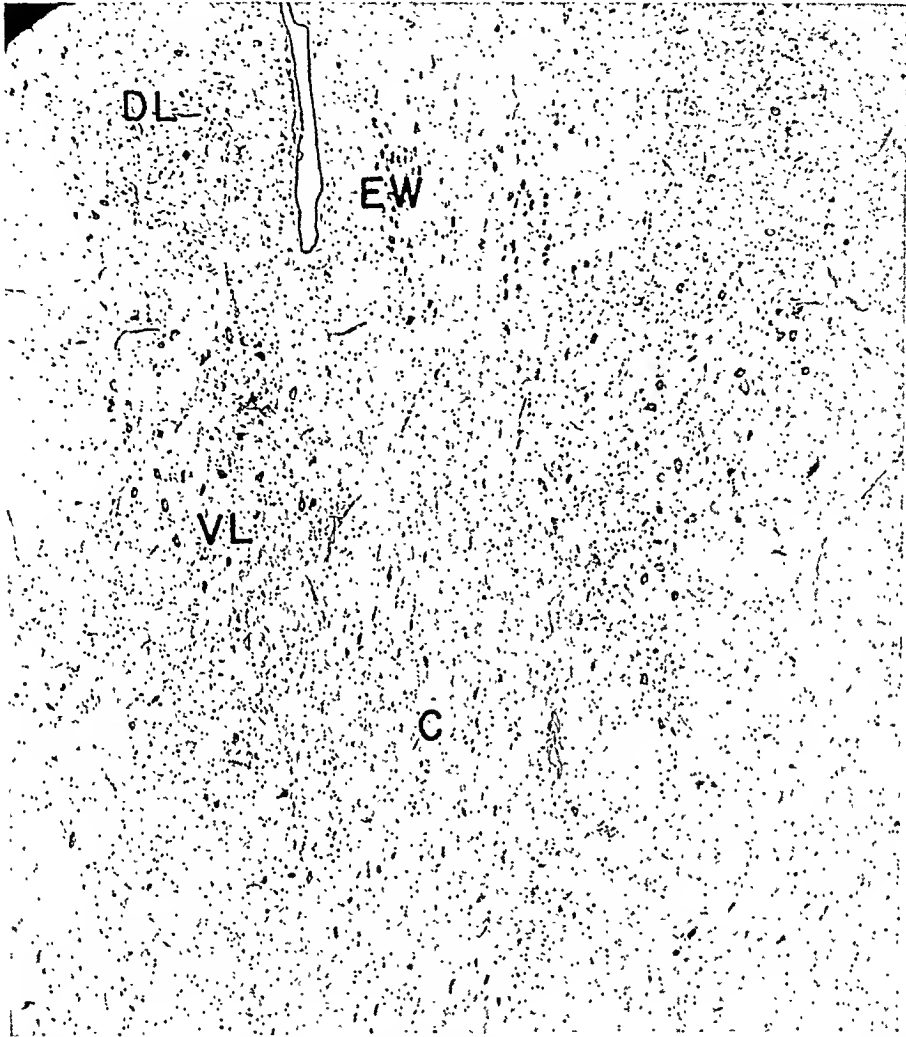


FIG. 4. The oculomotor nucleus, showing reduction in cells of various nuclei, particularly the dorsal lateral, ventral lateral, and central. C, central nucleus of Perlia; DL, dorsal lateral nucleus; EW, Edinger-Westphal nucleus; VL, ventral lateral nucleus. (Cresyl violet, $\times 45$.)

Examination of the remainder of the brain disclosed the following: Edema, perivascular in location, was prominent in many parts of the brain, especially in the white matter. In the oculomotor, trochlear, and abducent nuclei there was considerable dropping out of nerve cells, degenerative changes in many that remained, and moderate proliferation of microglia and oligodendroglia. A representative field from the oculomotor nucleus is illustrated in Fig. 4. Somewhat slighter changes were observed in the vestibular nuclei, especially in the medial vestibular nucleus. Nowhere in the cerebrum did the white matter show

abnormalities except for perivascular edema. No hemorrhages were observed. The other cerebral structures were in the realm of normal.

One section at the level of the pyramidal decussation showed nothing of significance. The remainder of the medulla oblongata was not available for study.

DISCUSSION

We are concerned here with a case of obvious pamaquine poisoning. The patient had also received quinacrine and quinine in standard dosage, but there had been no symptoms of intoxication up to the time pamaquine was administered. Altogether 1.2 gm. of pamaquine were received in one day; hence the patient was given 20 times the therapeutic dose if the dosage recommended by Goodman and Gilman (7) (0.06 gm. per day) is taken as the standard. In other fatal instances reported in the literature the amount of pamaquine given approximated the therapeutic dose. In the case of Blackie (2), the patient received the therapeutic dose, and in that of Cordes (6), 1.5 times the daily dose for 3 days. In the 2 fatal cases reported by Simeons (18) each of the patients had received only 0.02 gm. a day for 3 days. One wonders whether death in some of these cases is not to be ascribed to the black-water fever rather than to pamaquine intoxication. In view of the observation that quinacrine impedes the rate of disappearance of pamaquine from the blood plasma (Kennedy *et al.* (10)), it is possible that the quinacrine medication may indirectly have aggravated the pamaquine poisoning.

Despite the large dosage of pamaquine the pathologic changes in the central nervous system were few. The question arises as to the mechanism responsible for these changes. The cyanosis present from the onset reflected severe methemoglobinemia, and although the degree of methemoglobinemia was not determined during life, it was found to be 4 per cent at postmortem examination 7 days after the administration of pamaquine. Also there was hemoglobinuria for 3 days, but the anemia resulting from both methemoglobinemia and hemolysis may to some extent have been compensated for by the blood transfusions. The promptness with which the initial transfusion was given may well account for the delay in the fatal outcome and for the paucity of changes in the central nervous system.

The pattern of distribution of the cerebral changes is rather unique if the basic cause of the lesions is hypoxia incident to methemoglobinemia. For the basis pontis to bear the brunt of the attack in hypoxia whatever the cause, is decidedly uncommon. This part of the brain was not affected in cats subjected to bilateral carotid and subclavian ligature (Gomez and Pike (7)). Windle, Becker and Weil (22) observed pontile hemorrhage in guinea pigs, the umbilical cords of which were tied off while still in utero, but there was no evidence of hemorrhage in our case. Involvement of the globus pallidus, on the other hand, is commonly observed in anemic hypoxia, particularly after carbon monoxide poisoning, but in our case the damage to this structure was slight. The occurrence of ischemic changes in the nerve cells of the cerebral cortex is consistent with hypoxia.

Despite the paucity of changes it seems plausible to assume that death was due

to prolonged hypoxia and to complicating pneumonia. There were also bulbar symptoms (difficulty in articulation and bilateral paralysis), but their importance in the outcome could not be ascertained. Evidence of bulbar involvement was observed also by Blackie (2) and Chopra and Abdul Waked (5). In view of the liver necrosis reported by Cordes (6) in a fatal case of pamaquine poisoning, it should be emphasized that the liver in our case was in the realm of normal. And, finally, it is of interest, in view of the degenerative changes in the oculomotor, trochlear, abducent and vestibular nuclei in our case, that these nuclei were found by Schmidt and Schmidt (17) and Richter (16) to be sites of predilection to poisoning by various quinoline compounds, including pamaquine, in monkeys.

SUMMARY

1. This report is concerned with a case of pamaquine poisoning in which approximately 20 times the therapeutic dose of the drug was given in one day. Death occurred 7 days thereafter.

2. Outstanding early clinical symptoms included apprehension, cyanosis, nausea, and generalized pains; and late symptoms, numbness of the face, difficulty in speaking, dyspnea, and palatal paralysis.

3. Methemoglobinemia and hemoglobinuria occurred soon after the ingestion of the pamaquine.

4. From the standpoint of pathologic anatomy, there was ischemic necrosis with reactive change in a small area of the basis pontis, and mild to moderate degenerative changes in the globus pallidus, nuclei of the extraocular nerves, vestibular nuclei, and cerebral cortex. The outstanding visceral lesion consisted of lobular pneumonia.

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PARENTERAL USE OF CAMOQUIN HYDROCHLORIDE AS AN ANTIMALARIAL

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The authors recognize the need for a rapid acting parenteral antimalarial. Realizing that oral medication is the method of choice, there remains, however, many instances in which it is difficult or impossible for the patient to take drugs by mouth. Among these, we might mention the mental patient who is violent or contrary, the patient in coma suffering from pernicious malaria, or the patient who is vomiting.

Camoquin Hydrochloride⁴, (SN 10751), a new synthetic antimalarial, has been studied in order to determine its possibilities as a successful antimalarial for parenteral use. It is chemically known as 4(3'-diethylamino-methyl-4'-hydroxyanilino)-7-chloroquinoline dihydrochloride dihydrate. In crystalline form, it is light yellow in color and soluble in water in a 5 per cent concentration at room temperature. The synthesis of this drug was first reported at a meeting of the American Chemical Society in 1946 (1).

Various workers have checked the effectiveness of this drug against avian malaria. Gingrich found it 6 times as effective as quinine against *P. cathemerium* in the canary. Coatney reported it 8 times as effective as quinine against *P. gallinareum* in the chick, while Porter found it 25 times as effective. Marshall found it 15 times as effective as quinine against *P. lophurae* in the duck (2, 3).

Coggeshall (4), the first to use Camoquin in treatment of naturally acquired human malaria, selected a group of 100 men infected with South Pacific relapsing vivax malaria for his study. He found that 1.0 Gm. of Camoquin in twenty-four hours was sufficient to terminate an acute attack and that chronic cases became parasite-free and clinically asymptomatic after a dose of 0.5 Gm. weekly.

In a preliminary report by Simeons and Chhatre (5), based on observations in India (1946), malaria patients were treated with a single oral dose of Camoquin Hydrochloride varying from 5 mg./Kg. to 15 mg./Kg. These authors decided 10 mg./Kg. was the optimum dosage.

In a study by Halawani and associates (6), using divided dosage, *P. vivax* disappeared from the blood in some cases following administration of 50 mg. Camoquin Hydrochloride twice daily for two days, or a total of 0.2 Gm. In others, four days' treatment, or a total of 0.4 Gm., were required to produce a clinical cure. In another series, patients with benign tertian and malignant tertian malaria were given 0.15 Gm. twice daily for a period of two to four days, parasites disappeared from the blood within forty-eight hours.

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Mein and Rosado (7), in the Amazon Valley, and Penido, de Souza and Bezerra (8) in the Rio Doce Valley, conducted a series of controlled field studies in which they ran concurrent clinical trials of Camoquin and three other anti-malarials. They concluded from their results that Camoquin was the drug of choice. These two studies were conducted separately, and independently of each other; adequate blood studies and "follow-up" were included in both programs.

Mein and Deane reported at the International Congress of Tropical Medicine and Malaria (May 1948), Washington, D. C., that when a single dose of 0.5 Gm. Camoquin was used in the adult, the blood was cleared of circulating parasites, with the exception of the gametocytes in some *P. falciparum* infections.

Sister M. Mercy, M. D. (9) reported that Camoquin Hydrochloride cleared the blood of gametocytes in resistant *P. falciparum* infections if the dosage was increased to a total of 3 to 7 Gm. given over two and three days, respectively.

Camoquin Hydrochloride is distinguished by the rapidity with which it clears the blood of plasmodia according to these reports of Coggeshall, Simeons, Halawani, Sister Mercy, Mein and Penido. After a careful study of the results obtained by the above investigators, it was determined that the minimal effective dose of Camoquin which would interrupt the clinical course of malaria would be 0.3 to 0.35 Gm. of the base equivalent by mouth. Since this study concerned minimum effective dose, it was decided that for parenteral use the dose should be reduced to two doses of 0.123 mg. each of base equivalent, given six hours apart.

MATERIALS AND METHODS

Camoquin Hydrochloride in solution containing 0.031 Gm. of the base per cubic centimeter was placed in ampoules, each containing 2 cc. of the solution. Twenty-seven cases treated were paretic patients who had been given trophozoite induced malaria as a therapeutic measure. Both *P. vivax* and *P. malariae* were used. These strains appeared to be active and produced gametocytes in all patients inoculated. When each patient had undergone the specified number of rigors (5 to 15), Camoquin Hydrochloride solution was given intramuscularly in an amount equivalent to 0.123 Gm. of base. This dose was repeated in six hours. In case of recrudescence, this series of two doses was repeated.

Thick and thin smears were taken on each patient every few hours (every two hours when possible) from the first dose of the drug until the patients blood was repeatedly negative for parasites. Thick smears were then taken periodically on each patient for several months following treatment as a check on relapse.

RESULTS AND DISCUSSION

Table 1 presents in tabulated form the results obtained from treating patients inoculated with *P. vivax*. Table 2 presents a later series inoculated with *P. malariae*. The series of blood smears is not as complete as desired. The information on the length of time required to clear the blood, therefore, is not a com-

plete datum on all patients. The temperature charts of all patients were carefully followed and provided an accurate index to the clinical picture.

Temperature charts and progressive parasite counts are presented for 12 of the 27 patients of the series. Ten of the 12 patients were inoculated with *P. vivax* and 2 patients with *P. malariae*.

TABLE 1
Cases inoculated with P. vivax

PATIENT	AGE	SEX	INOCULATION TO FIRST ATTACK	FIRST ATTACK TO TREATMENT	TREATMENT	BLOOD NEGATIVE	RECRU- DESCENCE	RETREAT- MENT	FOLLOW-UP NEGATIVE
			<i>days</i>	<i>days</i>	<i>grams</i>	<i>hours</i>	<i>days</i>	<i>grams</i>	<i>days</i>
F. S.	41	F	8	7	0.246	120	0		240
F. J.	54	M	5	7	0.246	96	0		320
V. G.	53	M	6	30	0.246	192	40	0.246	230
					+				
					0.246				
A. C.	47	F	2 (?)	8	0.246	148	0		340
I. P.	49	F	8	10	0.246	(?)	0		360
E. H.	44	M	5	14	0.246	(?)	0		270
					+				
					0.246				
E. K.	34	F	11	11	0.186	96	22	0.123	330
I. G.	64	M	7	18	0.246	24	0		360
N. M.	41	M	18	14	0.246		15	0.246	210
					+			+	
					0.246			0.246	
C. J.	48	M	6	14	0.246	48	0		360
F. B.	42	F	10	15	0.246	36	0		240

After starting the study, it soon became evident that the total dose of 0.246 Gm. was on the borderline of the minimum effective dose. It was decided to continue with the same dose, however, treating each recrudescence as it appeared. It was felt that a parenteral antimalarial should have as its major therapeutic purpose, sufficient effectiveness to induce immediate remission of symptoms, this to be followed by a curative oral dose. For this purpose, the dose used was adequate even in those patients who manifested an early relapse.

Evidence adduced by this study indicates that the parenteral efficacy of Camoquin in induced malaria is approximately the same as when taken by the oral route.

The condition of one patient (F. J.) gave misleading information at the time of observation. At the time of the gluteal injection of Camoquin, the patient was developing an abscess from an anal fistula and, the resulting fever confused the fever curve.

Only one patient showed an induration following intramuscular injection; the induration was small in area and disappeared within thirty-six hours. With

this one exception, none of the patients exhibited any toxic reaction either locally or systemically.

TABLE 2
Cases inoculated with P. Malariae

PATIENT	AGE	SEX	INOCULATION TO FIRST ATTACK	FIRST ATTACK TO TREAT- MENT	TREAT- MENT	FEVER ENDED	RECRU- DESCENCE	RETREAT- MENT	FEVER ENDED	FOLLOW- UP NEGATIVE
			<i>days</i>	<i>days</i>	<i>grams</i>	<i>hours</i>	<i>days</i>	<i>grams</i>	<i>hours</i>	<i>days</i>
C. Mc.	45	M	10	29	0.246	24				240
B. N.	41	M	23	32	0.246	36				270
					+					
					0.246					
C. P.	47	M	17	14	0.246	24	11	0.246	36	180
								+		
								0.246		
H. B.	47	M	42	37	0.246	48	120	0.246	48	60
I. H.	34	M	?	?	0.246	48				180
F. K.	40	M	10	15	0.246	48				150
					+					
					0.246					
R. G.	37	F	13	15	0.246	48				90
I. M.	44	M	19	22	0.246	24				70
W. O.	59	M	16	15	0.246	132	34	0.246	48	30
R. S.	55	M	45	40	0.246	48				45
					+					
					0.246					
P. H.	30	M	18	23	0.246	48				45
H. P.	46	M	20	26	0.246	24				120
L. D.	37	M	21	20	0.246	24				90
					+					
					0.246					
L. W.	60	F	14	21	0.246	24				120
R. C.	40	M	17	10	0.246	24				120
					+					
					0.123					
R. S.	48	M	25	16	0.246	24	8	0.246	24	90

CONCLUSIONS

1. Camoquin Hydrochloride is an effective parenteral (intramuscular) drug for interrupting the clinical course of induced malaria.

2. Camoquin is free of untoward local reactions when given by intramuscular injection.

3. As an emergency measure in pernicious malaria, the parenteral administration of Camoquin should be of value for initiation of treatment.

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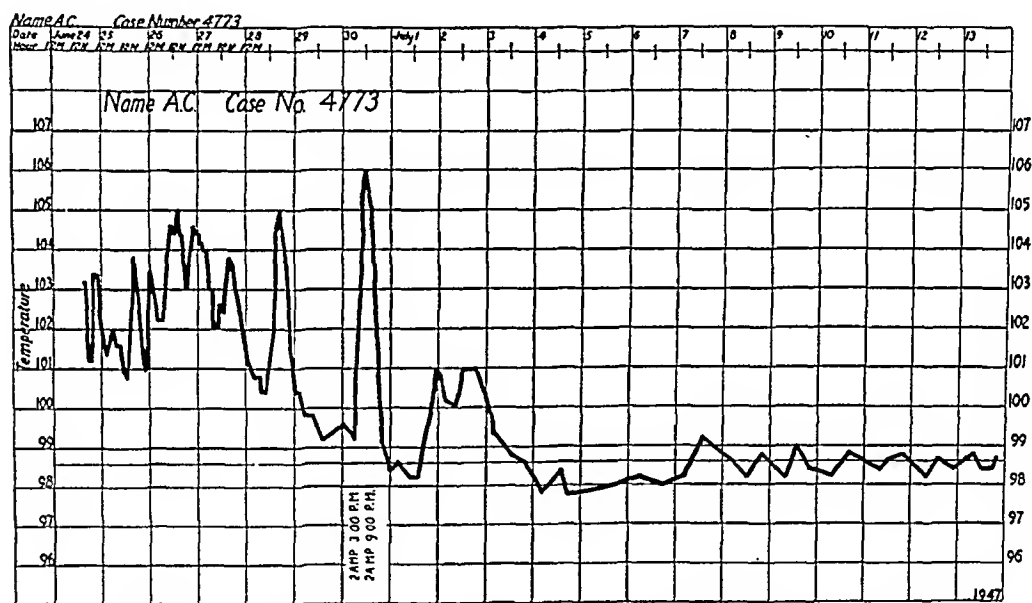


FIG. 1. Case: A. C. White female, 47 years. Inoculated *P. Vivax*: 6-22-47. First attack: 6-24-47. Type, Tertian

Date	Treatment	RBC Parasitized
6-30-47	3:00 P.M. 123 mg. I.M.	
	3:30 P.M.	0.01%
	5:00 P.M.	0.0004%
	9:00 P.M.	0.0001%
	9:00 P.M. 123 mg. I.M.	
7-1-47	8:00 A.M.	0.001%
7-5-47		Thick smear neg.
8-14-47	2:35 P.M.	Thick smear neg.
8-15-47	2:30 P.M.	Thick smear neg.
8-16-47	9:00 A.M.	Thick smear neg.
6-30-48	No relapses. No parasites found on follow-up.	

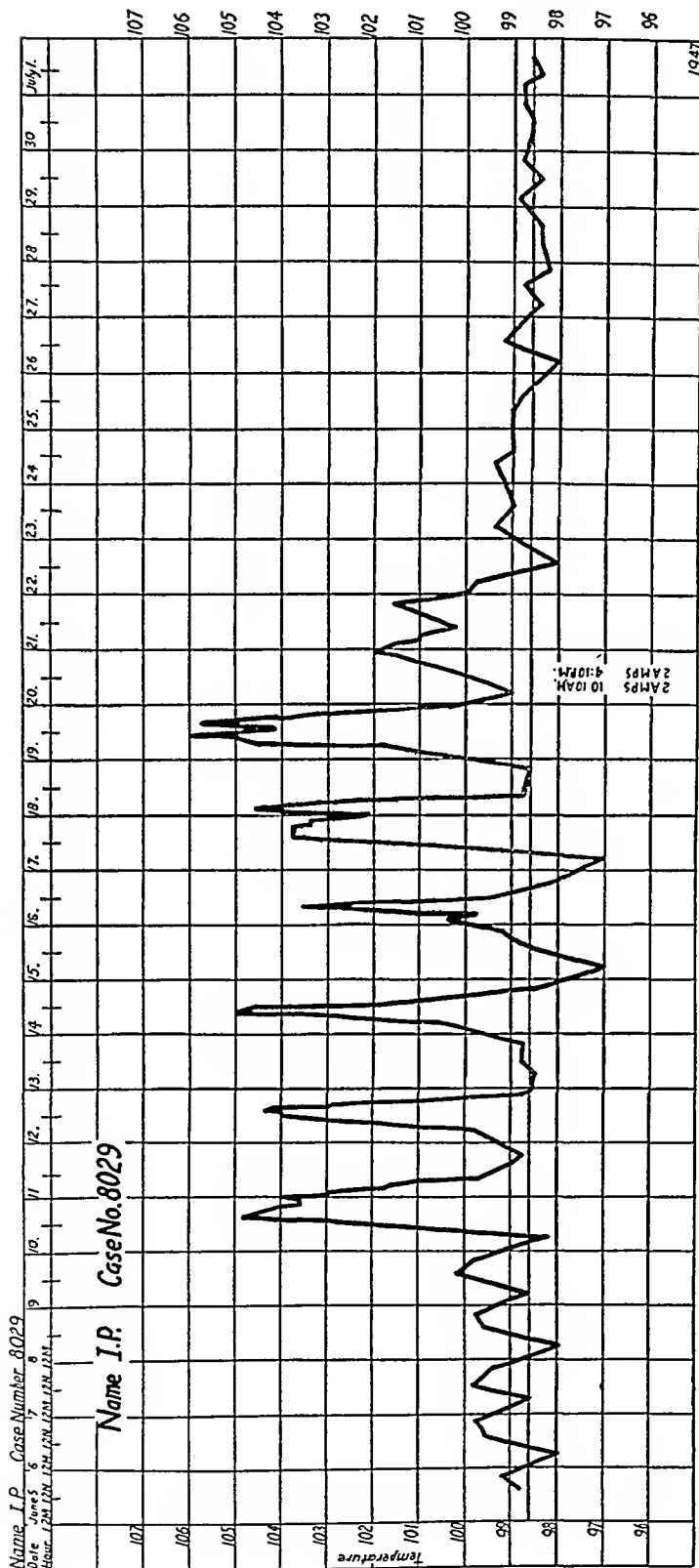


FIG. 3. Case: I. P. White female, 49 years. Inoculated *P. Vivax*: 6-2-47. First attack: 6-5-47. Type, Tertian

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
6-14-47	11:30 P.M.	0.11%	6-21-47	Smears lost.	
6-17-48	4:00 P.M.	0.34%	6-22-47	Smears lost.	
6-19-47	10:15 P.M.	0.54%	8-14-47	3:00 P.M.	Thick smear neg.
6-20-47	10:30 A.M.		8-15-47	3:30 P.M.	Thick smear neg.
	2:00 P.M.	0.17%	8-16-47	9:00 A.M.	Thick smear neg.
	4:20 P.M.				
	5:00 P.M.	0.47%	6-30-48	No relapses.	No parasites found on monthly follow-up.

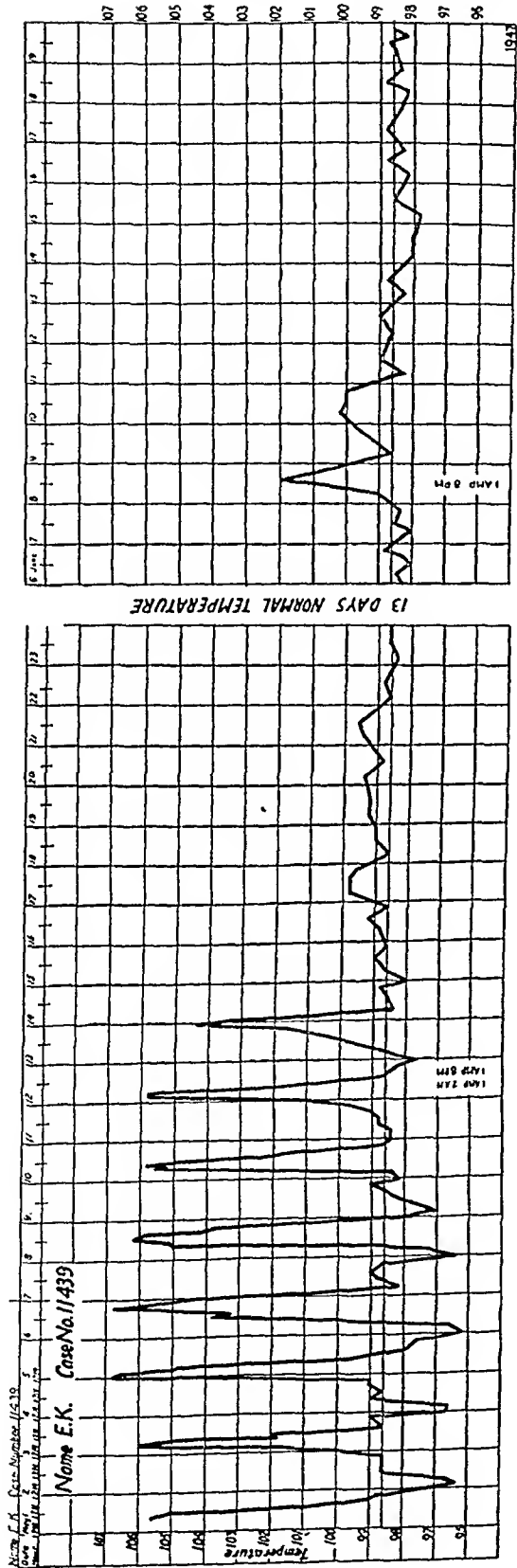


Fig. 4. Case: E. K. White female, 34 years. Inoculated *P. Vivax*: 4-19-47. First attack: 5-1-47. Type Tertian

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
5-1-47		0.07%	5-16-47	10:00 A.M.	Thick smear neg.
5-10-47		0.20%		A.M.	Thick smear neg.
5-12-47		0.40%	6-8-47	10:00 A.M.	0.19%
			8:00 P.M.	Recrudescence	
				62 mg.	
			6-9-47	2:00 P.M.	0.017%
				A.M.	0.003%
5-13-47		0.10%	8-14-47	5:00 P.M.	Thick smear neg.
		0.09%	8-15-47	3:30 P.M.	Thick smear neg.
		0.03%	8-16-47	9:00 A.M.	Thick smear neg.
5-14-47		Thick smear neg.	6-30-48		No further relapses. No parasites found in blood.

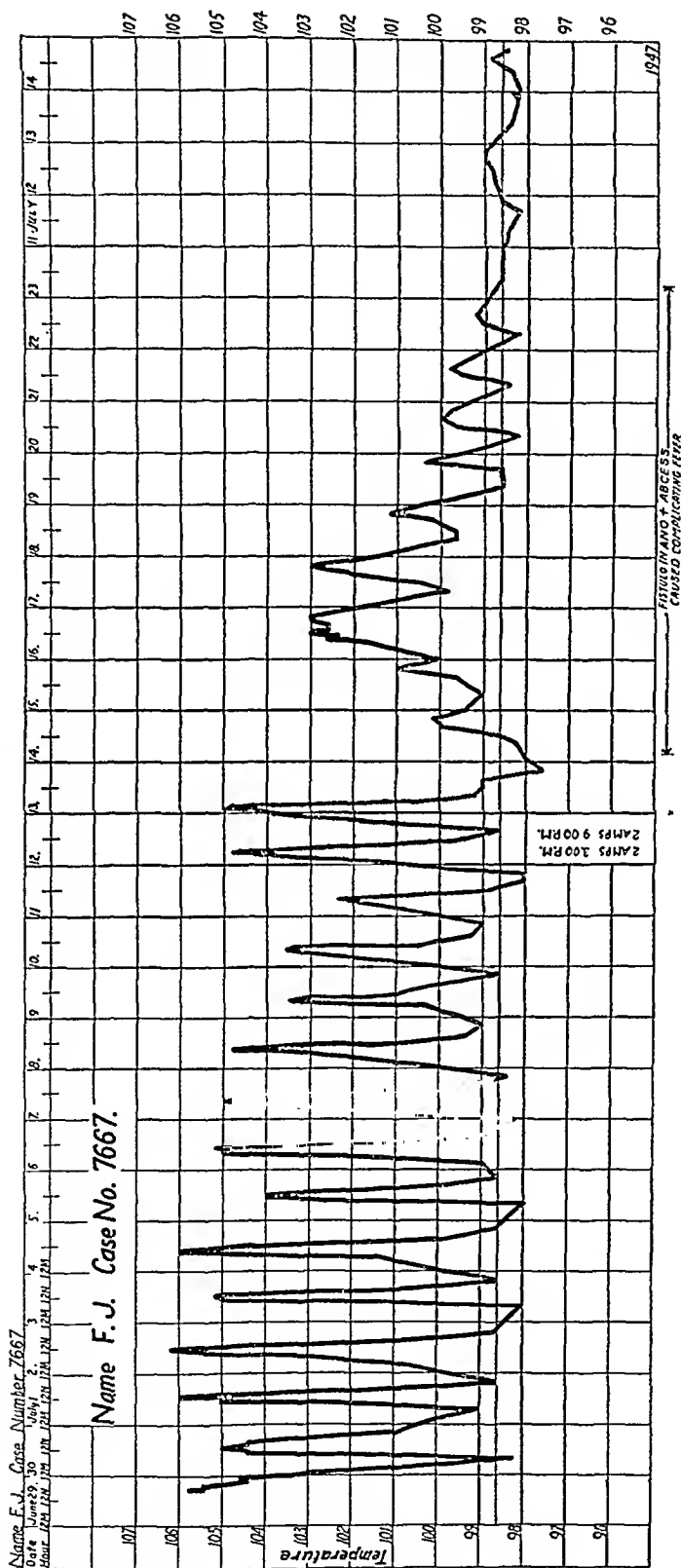


FIG. 5. Case: F. J. White male, 54 years. Inoculated *P. Vivax*: 7-1-47. First attack: 7-5-47. Type, Tertian

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
7-12-47	Pre-Treatment Slides Lost 3:00 P.M. 123 mg. I.M. 5:00 P.M. 7:00 P.M. 9:00 P.M. 123 mg. I.M. 11:30 A.M.	0.39% 0.54% 0.30% 0.0004% Thick smear neg.	7-17-47	3:30 P.M. 5:30 P.M. 7:30 P.M. 7:30 P.M.	Thick smear neg. Thick smear neg. Thick smear neg. Thick smear neg.
7-13-47 7-16-47			6-30-48	No parasites found on monthly thick smear follow-up.	

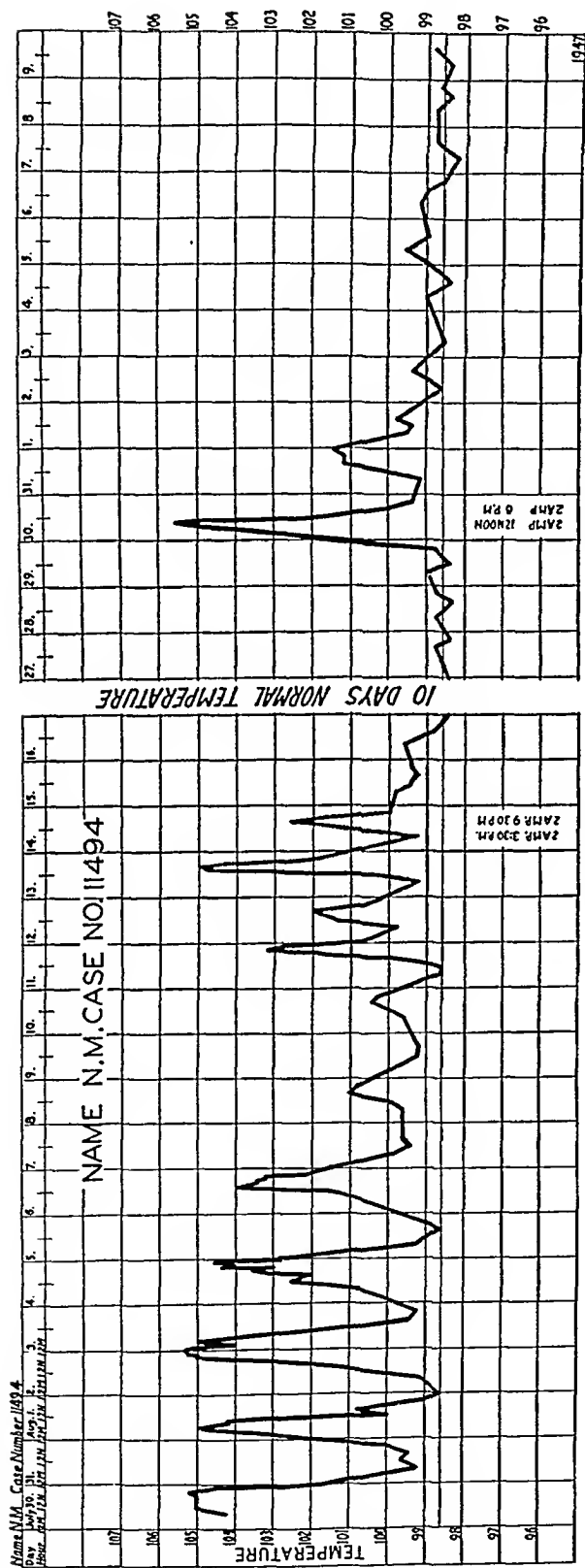


Fig. 7. Case: N. M. White male, 41 years. Inoculated *P. Vivax*: 7-12-47. First attack: 7-30-47. Type, Tertian

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
8-14-47	2:30 P.M.		10-12-47	8:00 P.M.	
	3:30 P.M.	123 mg.	10-16-47	10:00 A.M.	Recrudescence
	5:00 P.M.	I.M.	10-17-47	10:00 A.M.	
	7:30 P.M.			12:00 Noon	
	9:30 P.M.			2:00 P.M.	123 mg.
8-15-47	9:30 P.M.	123 mg.		8:00 P.M.	I.M.
	9:00 A.M.			8:00 A.M.	123 mg.
	10:30 A.M.		10-18-47	2:00 P.M.	I.M.
	12:00 Noon			1:30 P.M.	
	2:10 P.M.	123 mg.	10-23-47	10:00 A.M.	Thick smear neg.
8-30-47	4:00 P.M.	I.M.	10-24-47	2:00 P.M.	Thick smear neg.
	6:00 P.M.			4:00 P.M.	Thick smear neg.
	6:00 P.M.		10-28-47	4:00 P.M.	Thick smear neg.
	12:00 M.		11-26-47	4:00 P.M.	Thick smear neg.
10-2-47		0.01%	6-30-48	Thick smears remained negative.	
		Thick smear neg.			

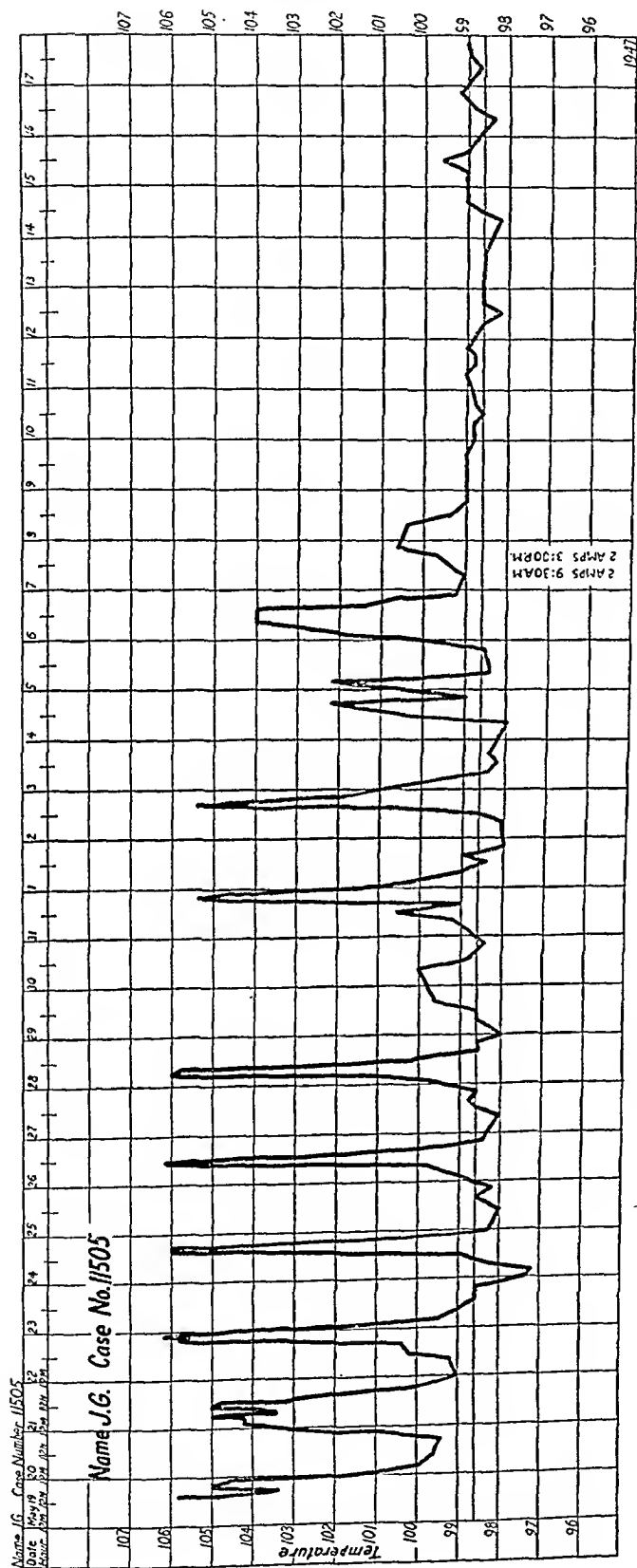


FIG. 8. Case: J. G. White male, 64 years. Inoculated *P. Vivax*: 5-12-47. First attack: 5-19-47. Type, Tertian

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
5-21-47	10:30 A.M.	0.5%	6-9-47	A.M.	Thick smear neg.
5-22-47	2:30 P.M.	0.009%	8-14-47	2:00 P.M.	Thick smear neg.
6-2-47		0.05%	8-15-47	2:30 P.M.	Thick smear neg.
6-7-47	9:30 A.M. 123 mg. I.M.	0.007%	8-16-47	4:00 P.M.	Thick smear neg.
	11:30 A.M.			9:00 A.M.	Thick smear neg.
	3:00 P.M. 123 mg. I.M.	0.004%			
6-8-47	12:00 Noon				
					No parasites found in blood on follow-up.

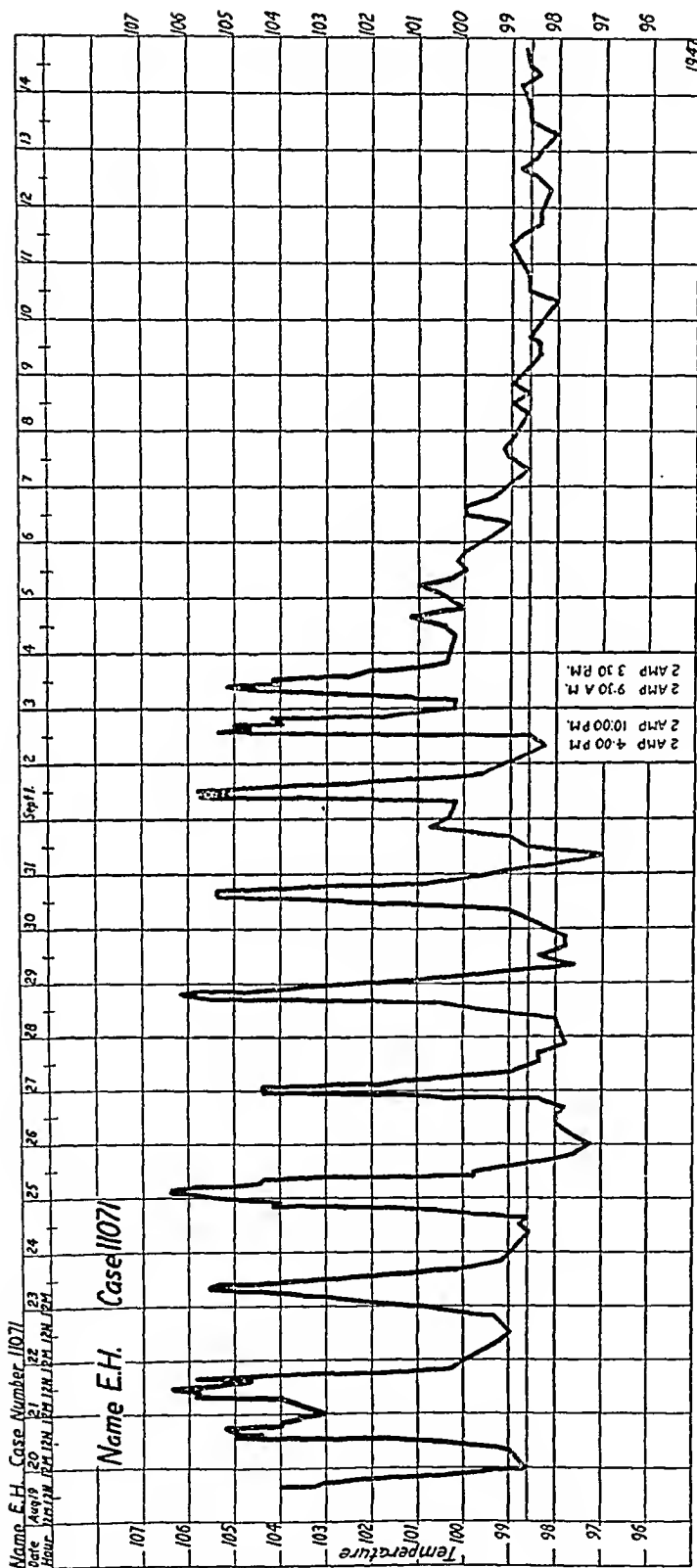


FIG. 9. Case: E. H. White male, 44 years. Inoculated *P. Vivax*: 8-14-47. First attack: 8-19-47. Type, Tertian

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
8-20-47	2:30 P.M.	0.3%	9-3-47	10:10 P.M. 123 mg. I.M.	0.06%
8-23-47	9:00 A.M.	0.08%		9:30 A.M. 123 mg. I.M.	0.04%
8-27-47	9:15 P.M.	0.4%		11:30 A.M.	0.02%
8-28-47	12:40 A.M.	0.07%		1:30 P.M.	Some induration on buttock
8-30-47	4:20 P.M.	0.01%		3:30 P.M. 123 mg. I.M.	0.02%
9-1-47	3:20 P.M.	0.006%			0.03%
9-1-47	9:45 A.M.	0.1%			Thick smear neg.
9-2-47	1:30 P.M.	0.1%	10-2-47	9:00 P.M.	
	4:00 P.M. 123 mg. I.M.		6-30-48		
	6:00 P.M.	0.15%			
	8:35 P.M.	0.08%			
					Follow-up negative.

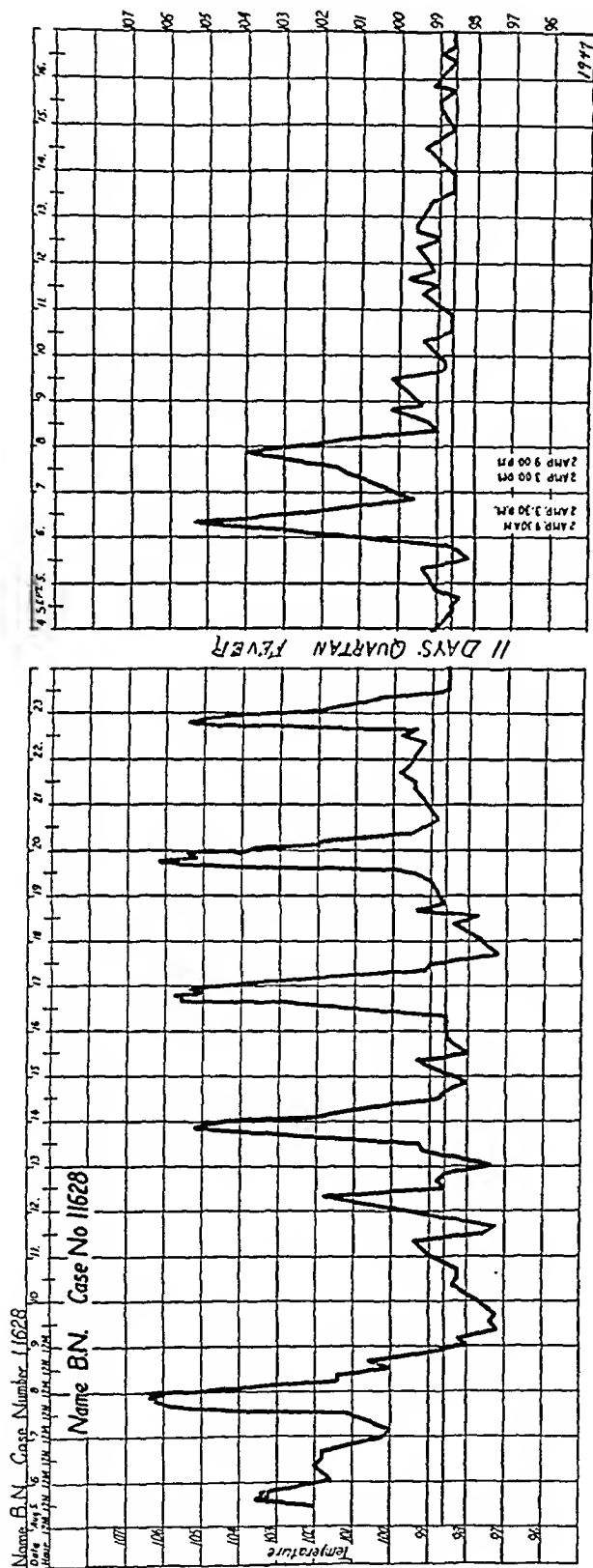
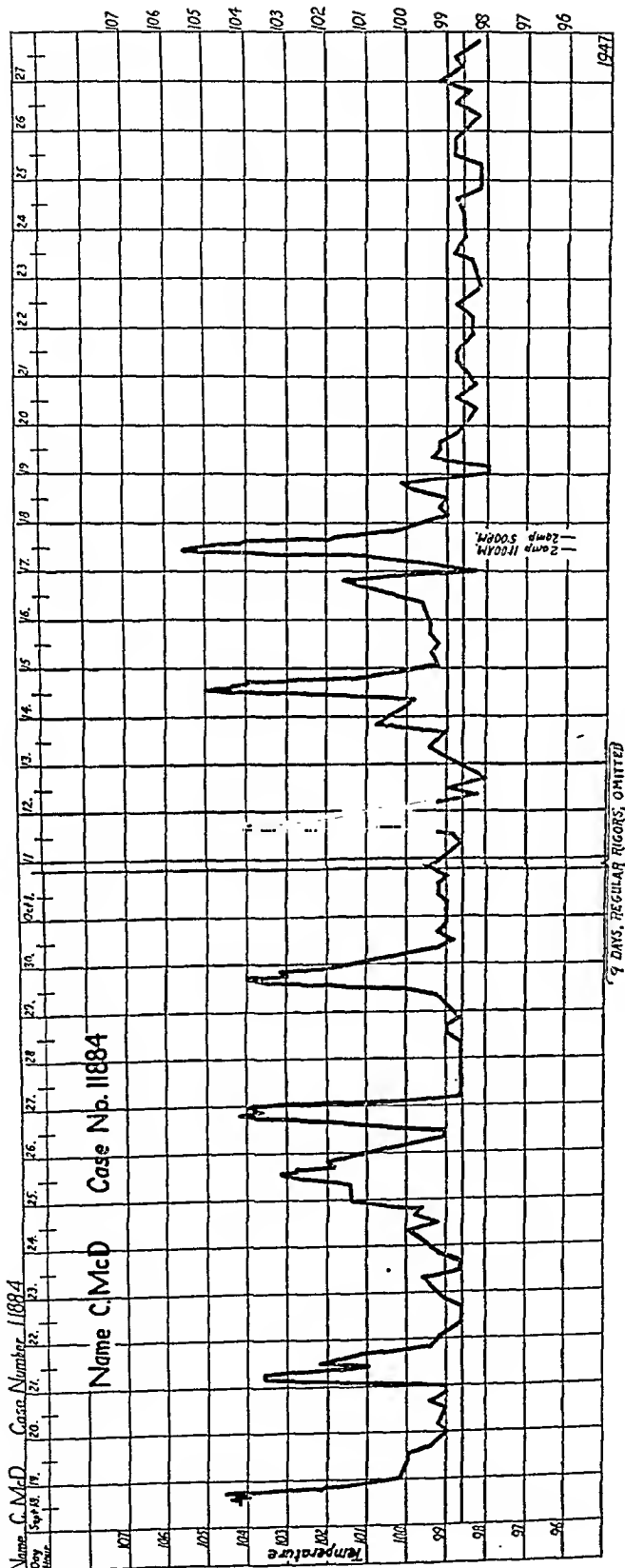


FIG. 11: Case: B. N. Black male, 41 years. Inoculated *P. Malariae*: 7-12-47. First attack: 8-5-47. Type, Quartan

Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
8-25-47	9:00 A.M.	0.25%	10-2-47	5:00 P.M.	Thick smear pos.
9-6-47	9:30 A.M. 123 mg. I.M.	0.004%		7:00 P.M.	0.0006%
	12:00 Noon	0.002%		9:00 P.M. 123 mg. I.M.	0.0006%
	1:30 P.M.	0.001%			Thick smear neg.
	3:30 P.M.	0.0007%			
9-7-47	9:00 P.M.	0.002%	6-30-48	Monthly thick smears negative.	
	3:00 P.M. 123 mg. I.M.	0.0005%			



Date	Treatment	RBC Parasitized	Date	Treatment	RBC Parasitized
9-18-47	3:00 P.M.	Thick smear neg.	10-23-47	3:00 P.M.	0.0003%
9-25-47	4:15 P.M.	0.0003%	11-25-47	5:00 P.M.	0.0003%
9-20-47		0.01%		5:00 P.M.	
10-2-47	8:30 A.M.	0.001%		10:00 P.M.	0.0008%
10-5-47	1:45 P.M.	0.0007%		3:30 P.M.	Thick smear neg.
10-16-47	10:00 A.M.	0.0005%			Thick smear neg.
10-17-47	11:00 A.M.	0.0009%	6-30-48	Monthly thick smears negative.	
	1:00 P.M.	0.0005%			

TRYPANOSOMA EQUIPERDUM, TRYPANOSOMA BRUCEI AND TRYPANOSOMA HIPPICUM INFECTIONS IN LABORATORY ANIMALS¹

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INTRODUCTION

During the past two decades the unprecedented developments in the field of chemotherapeutic agents, especially the introduction of the sulfonamides and antibiotics, have re-emphasized the importance of the use of *in vivo* methods in evaluation of recommended therapeutic substances. No *in vitro* methods have been developed which in any way compare with the biological means in producing conditions closely approximating natural occurring infections for such experimentation.

Parasites of the lower animals closely related to those which are pathogenic for man have been employed successfully in testing chemotherapeutic substances to be used in treatment of human infectious disease. A classical example of this is the plasmodium of avian malaria in the study of anti-malarials. In the related protozoan diseases, the trypanosomiases, infections in various laboratory animals with species of the flagellates non-pathogenic for man have been employed in a similar manner, although to a more limited degree. While the need for specific medicaments has not been so acute in connection with the latter group of diseases, it is none the less real.

The number of strains of trypanosomes maintained in laboratory collections is limited, since, for teaching purposes the non-pathogenic form, *T. lewisi*, is satisfactory and readily obtained from the wild rat and, not infrequently, from the white rat. Three parasitic species of known origin are available in this laboratory, *T. equiperdum*², *T. brucei*³ and *T. hippicum*⁴. All three organisms are the cause of fatal diseases of horses and are conveniently perpetuated in guinea pigs without danger to the personnel.

From a careful perusal of the literature it has been possible to find few reports of planned and controlled studies of the nature and progress of the infection in artificial hosts (Rouget, 1896; Nocard, 1900; Schneider and Buffard, 1900; Lingard, 1904; Bruce, 1895; Kanthack, Durham, and Blandford, 1898; Plimmer and Bradford, 1899; Plimmer and Bradford, 1901; Darling, 1910; Darling, 1911; Darling, 1912; Laveran, 1911; Pearce and Brown, 1918). The objective has

¹Based on data submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the University of Michigan.

²Secured from Dr. A. L. Tatum, University of Wisconsin.

³Lineally descended from the original isolation of Bruce, 1896. Obtained from Dr. Thomas, Liverpool School of Tropical Medicine about 1900.

⁴Culture secured from Dr. Herbert Clark, Gorgas Memorial Institute, Ancon, Canal Zone.

been, in the main, to maintain cultures for morphological studies and the course of the disease in the animal has been of secondary importance.

Because of this paucity of data an investigation was outlined to establish the three species of trypanosomes in various animals using infected guinea pig blood as a source of the organisms. With this accomplished, the course of the infection would be followed by regular examinations of the blood for parasites. On the basis of findings with bacteria and viruses, it was anticipated that several passages would be required in order to adapt the strains to new hosts. Nevertheless, it was hoped that a disease with a fixed clinical course or a so-called "standard infection" might result when all the techniques were carefully duplicated. If this followed, a tool would be available for biological assay of trypanocidal substances.

EXPERIMENTAL

The animals used were white rats, white mice, guinea pigs, rabbits, hamsters (*Cricetus auratus* Waterhouse), cotton rats (*Sigmodon hispidus minimus* and *Sigmodon hispidus cienaga*) and deer mice (*Peromyscus maniculatus*).

Infections with the three species of trypanosomes were produced in various animals by the injection of blood taken from guinea pigs in which the stock cultures were maintained. An effort was made to secure the blood for this initial inoculation at a time when it was rich in parasites. The organisms, once established by this method, were transferred from one animal to another through a series of from six to ten passages. All injections were made subcutaneously in the lower abdominal region.

For uniformity of procedure the inoculum in all instances was 0.5 ml. of a suspension made by adding two drops of infected blood to 1.0 ml. of sterile physiological salt solution (PSS 0.95 percent NaCl). With few exceptions the parasite count of the original blood was fifty or more per microscopic field (objective 1/12" or 96X, ocular 8X). The examinations were made with the aid of dark-field illumination at twenty-four hour intervals. Blood samples were taken from the ear of the rabbits and guinea pigs and from the tip of the tail in other species. Ten or more microscopic fields were studied and the average number of organisms present recorded.

The blood films were prepared as follows: A small quantity of the specimen, on a cover glass, was pressed against a glass slide; this provided a film which, while thick enough to allow free movement of the trypanosomes, was thin enough to prevent the red blood cells overlapping or being in layers. Thick films are objectionable because the alterations in surface tension at the edge of the film and pressure of the objective cause currents in the liquid. Such movement may mask detection of the flagellates, since their presence is usually revealed by the commotion among the red blood cells due to the activity of the organisms.

As an additional aid in following the course of the infection, and particularly the morphology of the trypanosomes, stained preparations were made of specimens at intervals. The films were air-dried, fixed in a mixture of equal parts

of ether and alcohol and stained by the Romanowsky technique as employed in this laboratory.

ROMANOWSKY STAIN

Solution A

Methylene blue.....	1.0 gram
Distilled water.....	50.0 ml.
Glycerine C.P.....	50.0 ml.
Sodium carbonate.....	0.25 gram

Dissolve sodium carbonate in distilled water. Add methylene blue and glycerine. Weigh. Heat in a beaker for 30 minutes at 90°C. Stir constantly. Add 5 ml. distilled water every 5 minutes and make up to original weight at the end of the heating process.

Solution B

Methylene blue.....	1.0 gram
Distilled water.....	100.0 ml.

Solution C

Eosin XBA.....	1.0 gram
Distilled water.....	100.0 ml.

PROCEDURE FOR STAINING BLOOD FILMS WITH ROMANOWSKY STAIN

Fix slides in ether-alcohol mixture 5 or more minutes. Air-dry. Place each slide in a staining dish which contains 20 ml. distilled water, 9 drops Solution A, 6 drops Solution B, and 3 drops Solution C which have been mixed thoroughly. Leave slide in stain 15 or 20 minutes. Wash in distilled water, dry and examine.

In certain instances the rectal temperature of the animals was determined before the blood specimen was taken. The temperature of the animals seemed to drop during the first thirty-six hours after inoculation, followed by a rise which persisted until death occurred. Changes in the temperature of the animal quarters which are known to affect the temperature of the animals played no part in the present observations.

All animals used in this study were full grown except the white rats. The experiments with this species were extended to include immature as well as old breeding stock. Lengthening of the incubation period and an increase in resistance seemed to be associated with the aging process, but there was only a day's difference in the time of death between the immature and the old animals.

The infections produced by the three species of organisms in white rats, white mice, cotton rats and hamsters were acute, progressive, fatal diseases characterized by the constant presence of the parasites in the blood. The incubation period, i.e. the time between inoculation and the appearance of the trypanosomes in the blood, was usually 48 to 84 hours and, once they had become established, continued to multiply until, in many instances, the number of trypanosomes exceeded that of the red blood cells. The parasites became less motile as the disease approached its termination and motile forms were not found at autopsy.

In stained preparations the trypanosomes showed vacuolation at the time motility began to decrease, and in specimens of blood taken shortly after death only disintegrating forms were found as anticipated from the direct examinations above. The nuclei and flagella of these fragmented forms retained their original staining characteristics.

Throughout the course of the infections there was little or no change in the general appearance of the animals or their reaction to stimuli until late in the disease when they became dull, sluggish, showed weakness and dyspnea. Any unusual disturbance at this time might precipitate death. In some other instances the animals went into paroxysms in which they seemed to be dying, yet survived for several hours.

The incubation period for the three trypanosomes was shortest for the white rat, and, in order of virulence for the rat, as well as for the other animals, the parasites were: *T. equiperdum*, *T. brucei*, *T. hippicum*.

No skin plaques or other external lesions were observed in the white rat, white mouse, hamster or cotton rat during the course of these trypanosome infections. At autopsy there appeared little change in the viscera except the enlargement and darkening of the spleen, (Perla, 1935) hemorrhages in the respiratory system and necrotic patches in the liver. (Andrews and Johnson, 1930; Linton, 1930).

In contrast with the above, the infections in rabbits, guinea pigs, and deer mice were of the subacute or chronic type. The blood of the rabbits rarely contained large numbers of trypanosomes. When present the organisms were large, sluggish forms similar to the pre-divisional forms found in other animals. As a rule the parasites were difficult to demonstrate and in many instances weeks elapsed with only negative findings. The symptoms of the infections were: edematous lesions about the ears, eyes and nose and on the skin of the body, respiratory involvement, shown by coughing and sneezing, and inflammation of the external genitalia.

In the initial stages of the infections the rabbits remained in fairly good condition, but as the disease progressed they became cachectic. In one instance, however, a rabbit infected with *T. hippicum* rallied from this emaciated state and appeared to have recovered except for lesions about the face and ears. No trypanosomes were found in the blood of the animal for twenty-six days before it was sacrificed on the 209th day after inoculation. At autopsy no trypanosomes were found in the heart's blood or in tissue smears from the spleen, kidney, lung or lymph node. No examination of the bone marrow was made, unfortunately.

The skin lesions occurred in rabbits in all three trypanosome infections. The organisms were present in these areas during the initial stages of their development. Later the lesions became dry and encrusted, with loss of hair. As the disease progressed the lesions about the face showed such marked suppuration that the animals were unable to open their eyes and the nasal passages were occluded.

In the guinea pig the three trypanosomes produced either subacute or chronic infections. The trypanosomes appeared in the blood three to seven days after inoculation and multiplied for a short period when they suddenly dropped in number or disappeared altogether from the peripheral circulation to reappear a few days later. This relapse phenomenon was repeated several times. In the few instances in which the disease was of long duration minor involvement of tissues occurred such as lesions about the eyes and swelling of the external

genitalia. At autopsy the viscera were little altered except for the enlargement and darkening of the spleen.

The *Peromyscus* mouse, like the rabbit and guinea pig, did not have the parasites circulating in the blood at all times. Relapses occurred but there seemed to be no particular regularity regarding this phenomenon. Unlike the rabbit, however, this animal at times showed tremendous numbers of trypanosomes in the blood, a condition true of all three species of trypanosomes.

TABLE I

Incubation period and duration of infection with T. equiperdum, T. brucei and T. hippicum in various laboratory animals

ANIMAL	INCUBATION PERIOD IN DAYS			DURATION OF INFECTION IN DAYS		
	<i>T. equiperdum</i>	<i>T. brucei</i>	<i>T. hippicum</i>	<i>T. equiperdum</i>	<i>T. brucei</i>	<i>T. hippicum</i>
White rat (mature).....	1.6	2.3	3.7	5.1	6.5	11.1
White rat (young).....	1.3	2.2	3.2	4.5	6.4	9.2
White mouse.....	2.2	3.1	3.7	5.0	7.4	10.0
Rabbit.....	2.5	10.0	27.5	29.0	38.0	112.0
Guinea pig.....	3.0	4.1	6.5	19.0	56.0	73.0
Hamster.....	2.0	3.5	3.0	7.5	14.0	12.5
Cotton rat.....	1.3	3.0	5.0	5.0	8.0	12.0
<i>Peromyscus</i> mouse.....	2.0	7.0	12.8	61.0	79.5	122.8

TABLE II

Influence of age of host on incubation period and duration of infections with T. equiperdum, T. brucei and T. hippicum in white rats

AGE OF ANIMALS	AVERAGE INCUBATION PERIOD IN DAYS			AVERAGE DURATION OF INFECTION IN DAYS		
	<i>T. equiperdum</i>	<i>T. brucei</i>	<i>T. hippicum</i>	<i>T. equiperdum</i>	<i>T. brucei</i>	<i>T. hippicum</i>
Old.....	1.5	2.2	2.3	4.3	6.5	7.0
Mature.....	1.3	2.0	1.5	4.0	6.4	7.0
Young.....	1.0	2.0	1.5	3.5	6.0	6.0

Five or six animals used in each instance.

The deer mouse was relatively resistant to infection with the trypanosomes, yet they finally succumbed to the diseases. The animals remained active and in apparently good condition until shortly before death. In the infections of particularly long duration the mice showed emaciation, but, up to the time of death, were capable of feverish activity when disturbed.

Tables I and II give the average incubation period and duration of the normal disease for the three trypanosome infections in the following animals: White rat, white mouse, rabbit, hamster, cotton rat and deer mouse.

DISCUSSION

In consideration of strains of trypanosomes which would be suitable for the development of standard infections in laboratory animals the three species, *T.*

equiperdum, *T. brucei* and *T. hippicum* as maintained in this laboratory seemed to be ideal. Such infections, in easily obtained experimental animals, would be an excellent tool for use in the evaluation of proposed trypanocidal drugs. *T. brucei* particularly recommends itself for such experimentation since evidence has been brought forward by some authorities to indicate that *T. gambiense* and *T. rhodesiense*, two of the most devastating of the trypanosomes pathogenic to man, are strains of *T. brucei* partially or wholly adapted to the human host. (Duke, 1924; Zavatarri, 1929; Duke, 1935) This parasite, as well as the other two, at the time of the investigation was fully adapted to the guinea pig, an experimental host, since the parasites are perpetuated in this species. None of these flagellates has had contact with its natural host for over fifteen years. *T. brucei* has been in the laboratory collection almost fifty years.

These three strains of organisms have many characteristics in common and several which are strikingly different. Morphologically they are very similar. They cannot be cultured on laboratory media which support the luxuriant growth of many species of trypanosomes. All may be transferred to various animals under laboratory conditions. In nature the three strains are associated with fatal endemic diseases of horses and occasionally of cattle and mules.

No difficulties were encountered in producing primary infections in the white rat, white mouse, guinea pig, rabbit, hamster, cotton rat or deer mouse by subcutaneous injection of infected guinea pig blood. There was considerable variation in the incubation periods of the strains in each host. With the establishment of the parasites in these animals each form was then passed in series through a sufficient number of transfers in each species of animal to adapt the strain to the host. Where an acute infection resulted, a period of adaptation was observed. The acute infections were characterized by a well-defined incubation period and a diseased state terminating in death.

When all the conditions which influenced the infections were standardized, the number of parasites in the blood at each stage of the disease was quite definite and predictable and thus became an index of the progress of the infection. It is believed that the white rat, white mouse and hamster under these conditions would serve ideally in an *in vivo* method for the assay of the trypanocidal action of chemical substances. In addition to prevention of death, a technique so often used in such experimental studies, the variations in the parasite count of the blood could be ascribed to the action of the drug. While it is dangerous to predicate on the basis of analogy, particularly when dealing with infection in such widely different hosts as the white rat and mouse and man, it is believed that the application of the second technique would be of great aid in the search for a specific drug for the treatment of the human trypanosomiasis.

The three species of trypanosomes produced fatal infections in the *Peromyscus* mouse, but the infection simulated the course of the disease in rabbits rather than the acute infection characteristic of the white mouse. The incubation period varied considerably in the deer mouse but the conspicuous feature was the undulating nature of the appearance of the parasites in the blood; for several successive days the number of organisms would exceed the number of red blood

cells, then the parasites would completely disappear, to reappear and disappear without apparent influence on the well-being of the host. Such circumstances would point to the possibility of the *Peromyscus* mouse playing an important role as a natural reservoir of these species of trypanosomes. It would be desirable to carefully investigate this possibility in areas where the diseases in horses and deer mice or allied genera coexist.

One of the characteristic changes associated with trypanosomes in horses and other large animals is an increase in temperature. This is of considerable diagnostic importance as well as an aid in following the course of the infection. In the present study the rectal temperature was taken at intervals of twelve hours. In the acute, fatal disease there was an increase in the temperature of the rat resulting from the presence of the trypanosomes, but it is doubtful that this elevation would be of diagnostic value as in the larger animals.

Serial transfer of the trypanosomes in young and mature rats suggested some natural immunity associated with the ageing process, however, inclusion of old breeding stock in the experiments failed to reveal conspicuous difference in incubation period or duration of the infections.

It is well to call attention to the striking difference in these trypanosome infections and the findings at autopsy in other infectious diseases. In instances where death is the result of such agents as viruses, bacteria, rickettsiae or higher fungi, the germ may be recovered from cadaveric tissue by cultural methods or animal inoculation. However, in the present study the parasites in the blood stream, just prior to death of the animal, were inactive, vacuolated and in many instances disintegrated. It was impossible to induce infection in normal animals by the injection of this blood or with postmortem material. It is possible that this phenomenon is the result of changes in the tissues of the host inimical to the survival of the parasite or the presence of a specific trypanocidal substance. Additional information on this subject would be of considerable value.

SUMMARY AND CONCLUSIONS

Infections with *T. equiperdum*, *T. brucei* and *T. hippicum* have been produced in white rats, white mice, rabbits, hamsters, cotton rats, guinea pigs and deer mice. When the inoculum was standardized as to volume, number and age of parasites and route of injection, the incubation periods were as follows:

	<i>T.</i> <i>equiperdum</i> (days)	<i>T.</i> <i>brucei</i> (days)	<i>T.</i> <i>hippicum</i> (days)
White rat (mature).....	2	3	3
White rat (young).....	1	3	3
White mouse.....	1	3	3
Guinea pig.....	3	5	9
Rabbit.....	3	10	28
Hamster.....	3	4	4
Cotton rat.....	2	2	4
<i>Peromyscus</i> mouse.....	2	6	10

The three strains of trypanosomes induced the same general symptoms in each species of the experimental hosts. The disease in the white rat, white

mouse, hamster and cotton rat was an acute, progressive, fatal infection accompanied by a uniform increase in the parasite count of the blood.

Rabbits showed an unusual involvement of the respiratory tract, inflammation of the external genitalia and edematous lesions at various places on the skin, particularly about the eyes, ears and nose. Trypanosomes could be found in these areas in the early stages of their development, later the lesions became encrusted and broke down and the trypanosomes disappeared. The animals survived for periods of one to four months during which time the organisms were rarely found in the blood.

In the guinea pig there were no outward manifestations of the disease until shortly before death. The parasite count of the blood fluctuated within wide limits and the so-called relapse phenomenon was frequently observed.

The trypanosomes produced a chronic infection in the deer mouse. The animals survived, in some instances, more than three months. As in the guinea pig, the parasites varied from day to day, on occasions blood specimens examined daily for more than a week would be negative, to be followed by specimens rich in organisms. In all the infected hosts, with the exception of the rabbit, the parasites were small, slender, active forms at the time of their appearance in the blood. As the disease progressed they increased in size with many dividing stages present. In acute infections they became vacuolated, agglutinated, and at the time of the death of the animal no actively motile forms were found.

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TRYPANOSOMA EQUIPERDUM, TRYPANOSOMA BRUCEI AND TRYPANOSOMA HIPPICUM INFECTIONS IN AVIAN HOSTS¹

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INTRODUCTION

It was during a search for a mammalian tool which could be used in *in vivo* assay of proposed trypanocidal drugs that the possibility of using chick embryos and chickens for such a purpose suggested itself. (Hood, 1949). For many years information has been available that mature fowls could be infected with trypanosomes only with great difficulty, if at all, and although, for almost two decades, it has been known that the chick embryo is a favorable medium for the cultivation of these parasites, little or no use has been made of this fact in the study of them.

In the early 1930's when the enthusiasm for the use of the chick embryo for cultivation of various forms of microbic life was at its height, several workers in this laboratory used the developing chick as a substrate in attempting to grow the various strains of spirochetes and trypanosomes maintained in the stock culture collection. The results varied. In some instances strains were passed through several generations by this procedure, but no well planned investigation was outlined to explore its full possibilities.

In the field of microbic research the chick embryo has been used with considerable success in the primary isolation and cultivation, evaluation of chemotherapeutic substances, titring of antibodies, production of anigens and a study of tissue changes with various species of viruses, rickettsiae and bacteria. The widespread employment of the developing chick embryo suggested an intensive investigation with trypanosomes. Accordingly such a study was outlined.

Techniques were perfected for the serial cultivation of trypanosomes in the developing embryos and strains of trypanosomes so adapted were injected into chickens with the hope of establishing the infection in that host. The details follow.

EXPERIMENTAL

In this series of experiments a modification of the method of Woodruff and Goodpasture (1931) was used in establishing *T. equiperdum*, *T. brucei* and *T. hippicum* in chick embryos. In the initial studies the eggs were obtained locally from two sources engaged in production for custom hatching. The eggs as delivered to the laboratory were placed in an incubator (Oakes) at 103° F. and turned twice daily. They were candled on the seventh day and those eggs in which the embryos had not developed were discarded. About sixty percent of

¹Based on data submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in the University of Michigan.

the eggs gave embryos which were satisfactory for use on the tenth day. The usual seasonal fluctuations in the fertility of eggs was observed. As the work progressed better results were obtained with eggs which had been incubated for seven days by the hatchery. This eliminated the exposure of the eggs to changes in temperature and humidity connected with the frequent opening and closing of the laboratory incubator doors.

After an incubation period of ten to twelve days at 103° F. the eggs were examined by candling to determine the position of the large blood vessels in the chorion overlying the yolk. A circle was marked on the shell with a lead pencil to denote the general area where the opening was to be made and lines were drawn across the circle to indicate the location of the blood vessels.

The area of the shell marked by the circle and lines was painted with tincture of iodine and allowed to stand for a few minutes in preparation for the next step. A small rectangular opening about $\frac{5}{16}$ " x $\frac{1}{2}$ " was cut in the shell with a rotary cutting tool fitted with a carborundum disc. Precaution was taken to keep the underlying membranes intact. The tip of a pair of sharp, curved forceps was pressed against the edge of the cut and the section of shell removed.

A layer of sterile paraffin oil was spread over the surface of the exposed membrane to aid in separation of the outer and inner tissues and to wipe away or fix bits of loosened shell. A small puncture in the outer membrane with the tip of the forceps allowed air to enter. Usually the spreading of the air space caused the chorion to drop, if it did not, forceps were used to lift the shell membrane and cut it away to expose the chorion, with its network of blood vessels, lying over the yolk. The embryo was then ready for inoculation.

The initial transfer was made with a saline suspension of heart's blood of a guinea pig infected with trypanosomes. This suspension containing one part of blood to about three parts of PSS, depending on the number of trypanosomes present. All subsequent inocula were prepared as follows: When the examination of the blood showed a high parasite count the cover glass was removed from the opening and, with the cutting tool, the shell was cut from one side of the opening, around the egg to the other side. With sterile forceps the halves of the shell were separated and the entire contents were transferred to a sterile Petri dish. The embryo was shifted very carefully to such a position that the large blood vessels could be opened to allow the blood to mix with the fluids escaping from the membranes. Extreme precaution was exercised to keep the yolk intact. The blood, diluted as indicated above, served as the inoculum. The blood of the embryo clots very quickly unless promptly mixed with the fluids. If clots occurred they were broken up to allow a sufficient number of organisms to escape to provide a satisfactory inoculum.

The amount of material introduced was 0.2 ml. This was drawn into a sterile syringe through a 26 gauge needle and subsequently injected into the yolk sac following a quick thrust of the needle to insure penetration of the enclosing membranes. If the surface of the yolk sac rose when the needle was lifted slightly the entrance was considered successful. It was necessary to inoculate the yolk, otherwise the incubation period was lengthened or the infection failed to develop.

The needle was withdrawn and the opening in the shell encircled with petrolatum containing three percent phenol and a cover glass superimposed to provide an hermetic seal. In this manner it was customary to inoculate six to nine embryos with each strain of trypanosomes at the beginning of each experiment. Incubation of the eggs was continued at 103° F. Daily after the fourth day following inoculation one of the eggs was opened aseptically, the contents transferred to a sterile Petri dish, and the blood examined for trypanosomes. If the parasite count was approximately twenty-five per microscopic field transfers were made as usual to fresh embryos, otherwise the material was discarded. Thus each embryo served for only one examination.

It was discovered, however, in the course of making the serial transfers, that the blood could be secured repeatedly without apparent injury by the following technique. The eggs were candled to determine the position of the large blood vessels. An area of the shell overlying a portion of the membranes which had no large veins was painted with iodine solution, then, with the egg under the candler, a nick was made in the shell with the rotary cutting tool previously treated with the iodine solution to prevent contamination. The cut was large enough to injure a few capillaries thus allowing the escape of a small quantity of blood. Much less than a drop was needed for this purpose. A cover glass was touched to the blood and a thin preparation made. The cuts in the shell usually closed immediately by the formation of a clot; if they did not, a small amount of melted sterile paraffin was placed over the opening to prevent desiccation.

With careful attention to asepsis it was found possible, in this manner, to follow the progress of the infection for a week or more by daily examination of the blood. In some eggs checked seven or eight times the chick continued to develop and even hatched, apparently unharmed by the many cuts which had been made in the shell or from the small quantities of blood lost during the sampling process. By this method it was possible, without opening the shell, to determine the density of the blood stream infection and the proper time to sacrifice the embryo to secure the desired inoculum. It was found that the trypanosomes established themselves in the embryo, and the blood with the desired number of organisms, was ready for transfer in the case of *T. equiperdum* in from four to seven days, *T. brucei*, in five to eight days, while *T. hippicum* required as much as six to nine days.

Since the method of sampling the blood to follow the course of the infection permitted the continued development of the embryo, the excess of eggs now available, because six to nine were inoculated at the beginning of each experiment, were left in the incubator to ascertain whether or not the infection would be maintained up to and after the emerging of the fully developed chick. It will be recalled that the method of inoculating included the making of a glass covered window. This precluded the proper handling of the eggs to insure well developed chicks, hence another method of inoculation was developed.

Eggs, incubated for the proper length of time, were candled as before to locate the yolk and the large blood vessels. Then, with the egg still under the candler,

the spot selected was painted with iodine solution and a cut, just large enough to admit a needle, was made in the shell. The 0.2 ml. of inoculum, prepared as previously described, was introduced into the yolk sac. The cut in the shell was closed with melted paraffin as usual. For the remaining portion of the twenty-one day period of incubation the eggs were kept in regular incubator trays at 103° F. and turned twice daily. Attention should be called to the fact that this new method of inoculation which was developed during the serial transfer experiments replaced the earlier method.

The parasites were carried through six serial passages over periods of twenty-six, thirty and thirty-two days respectively, when the experiment was terminated since it was clear that with the newly developed techniques there was every reason to believe that the strains could be perpetuated indefinitely in this manner.

As mentioned earlier, those embryos which were not used for the serial transfers were incubated the full twenty-one days. In order to insure their development it was found most satisfactory to inoculate the embryos on the twelfth to fourteenth day of their incubation period. Many embryos, weakened by the infection, were unable to complete their emergence from the shell, while others died before the termination of the incubation period. Special effort was made to facilitate their hatching.

The blood of the developing embryos was examined daily for the presence of parasites and blood studies were made on those which emerged from the shell. It was observed that the yolk sac of those infected chicks which hatched usually was not absorbed for several days. The blood films from newly hatched chicks were made from samples taken from the tip of the toe after cutting the nail with sharp scissors. In older chickens the blood was secured readily from one of the wing veins in the customary manner.

The trypanosomes usually disappeared from the blood of the chick within forty-eight hours after hatching and did not reappear. In those instances in which the parasites did persist for more than forty-eight hours, the chicks ate little, if at all, became weak and emaciated, seemed chilled and sleepy, and, when disturbed or handled appeared very uncomfortable. As the disease progressed, the feet became swollen and the chicks soon ceased to try to stand. Within four to six days death occurred, accompanied by intestinal hemorrhage, and in a few instances, the contents of the partially absorbed yolk sac erupted on the outside of the body. In only one instance, in an infection with *T. equiperdum*, did the parasites persist longer than two days and the chick recover. In this case the trypanosomes were present in the blood until the sixth day.

Those chicks which overcame the infection displayed no apparent ill effects from having harbored the trypanosomes. The parasites became vacuolated and lost motility by the second day, and shortly began to agglutinate and become non-motile. During the next twenty-four hours all traces of the trypanosomes disappeared and blood examinations made daily during the next week were negative. These chicks were checked for chronic infections after six to eight weeks by the inoculation of their blood into mice. No trypanosomes were demonstrated in the blood of these mice at any time during the fifteen-day period in which examinations were made at three-day intervals.

During the various experiments dealing with the establishment of the three strains of trypanosomes in chicks and chick embryos no morphological changes were observed, either by direct microscopic examination or by stained preparations. The same sequence of developmental forms appeared in the serial cultures in the embryo as was observed in mammalian hosts.

After the parasites had established themselves in the embryo they increased in number until the infection had reached its height. The embryos usually hatched with a large number of trypanosomes in their blood or died before the end of the incubation period. Out of more than fifty chicks hatched in only two instances did chicks, which had had trypanosomes in their blood, hatch free of parasites. There had been only a light infection and few organisms were found in the films made from them while still in the shell.

As had been anticipated, since the interval occurring between the time of inoculation and the termination of the incubation period was of such short duration, there was no suggestion of the relapse phenomenon in chick embryos such as was observed in the rabbit, guinea pig and *Peromyscus* mouse infected with the three strains of trypanosomes.

The question of the establishment of trypanosome infections in various fowls with the strains of organisms from mammalian sources has been frequently subjected to laboratory experimentation. (Bruce, 1895; Rouget, 1896; Ziemann, 1902; Goebel, 1906; Mesnil and Martin, 1906; Corson, 1931; Corson, 1932; Clark and Dunn, 1933; Mesnil, Leger, and Perard, 1936; and Seager, 1944). There are occasional references to the production of transient infections in chickens and other species with these and other strains.

In the present work three sources of infected material were (1) blood, from guinea pigs, rich in trypanosomes, (2) blood from passage strains in chick embryos, and (3) infected blood from chicks. The normal healthy chicks, raised in the laboratory, or procured from a local hatchery, were inoculated when three to seven days of age.

The infected guinea pig blood was obtained from animals in which the laboratory strains of trypanosomes were being perpetuated. A droplet or two of blood was taken from the ear vein of the guinea pig, mixed with saline solution in the barrel of the syringe and the suspension injected subcutaneously into the thigh area of several chickens in each experiment. A specimen of blood was taken from the wing vein every third day for four weeks after which the examinations were made at weekly intervals for eight weeks more. During this period no trypanosomes were found by examination of blood films made from these chickens. At the end of twelve weeks the chickens were sacrificed and three mice were injected with 0.5 ml. of a saline suspension prepared from each chicken, one with a dilution of blood, one with an emulsion of brain tissue, while the third received a suspension of macerated spleen. It was hoped that these injections might make it possible to detect chronic infection in which the number of parasites was so low that it was impossible to demonstrate them microscopically, as well as giving a clue to their point of localization in the body of the host. In only one instance was an infection of this sort detected. A mouse injected with blood from a chicken which had received twelve weeks earlier

guinea pig blood infected with *T. equiperdum*, developed a typical blood infection with *T. equiperdum*, although the incubation period was somewhat protracted. Mice injected with brain tissue and macerated spleen from this same chicken remained free from infection. The extent of the blood stream infection of the chicken at the time of the mouse injection was below the level detectable by microscopic examination. This positive result is somewhat enigmatical.

The embryo passage material used for the injection of the chickens was obtained in the same manner as described for making serial passages. Only first or second transfer cultures were injected into the chickens. This is open to the criticism that the strains were not adequately adapted to the new medium. Again, in only one instance, and again with *T. equiperdum* were the results positive, and as before, this infection in the chicken was detected only when the blood was injected into a mouse and not by examination of the blood.

Chicks which hatched infected with *T. brucei* and *T. hippicum* as mentioned earlier, either overcame the infection within forty-eight hours or succumbed within four to six days. In attempting to establish the parasites from these chicks in normal chicks, blood taken from the heart of those with infections which would have terminated shortly, was mixed with saline and injected subcutaneously into normal chicks. Tests for evidence of infection were made as already described. All findings were negative. No material was available for experimentation with *T. equiperdum* since the embryos usually died when infected with this form. One exception occurred, however. In the blood of one chick which was heavily infected when hatched the infection persisted for five days and then disappeared.

These negative data which are confirmatory of the work of others are somewhat disappointing since there was some reason to believe that the adaptation of the trypanosomes to the chick embryo as a culture medium might have enabled such strains to infect normal healthy chicks.

DISCUSSION

The cultivation of *T. equiperdum*, *T. brucei* and *T. hippicum* on laboratory media such as blood agar of Novy, MacNeal and Nicolle has been attempted on many occasions and by well-trained investigators. There are occasional reports of success and many accounts of failures. On inquiry at type culture collections, or among specialists in this discipline, such cultures are not available. It is clear from the description of these cultures that the morphology of the cells is not identical with the tissue-invading stage. Nevertheless cultures would be an inexpensive and convenient method of handling the organisms in the laboratory. These facts emphasize the need for a living substrate on which the typical forms of trypanosomes will develop.

The publication of Woodruff and Goodpasture called attention to the possibility of the chick embryo's serving as a medium for the cultivation of agents of disease, and, in certain fields, extensive use has been made of the developing chick for this and other purposes.

A few investigators have employed the chick embryo in experimental studies

with trypanosomes. Oag, in 1940, secured a few initial infections in eggs inoculated with *T. brucei*. Longley, Clausen and Tatum (1939) used this medium in an effort to alter drug fastness of *T. rhodesiense*. These workers succeeded in carrying the parasites through eight passages in forty-one days, using a modification of Goodpasture's technique. Altire-Werber (1941), in an attempt to determine the optimum number of trypanosomes which should be introduced to establish them in the chick embryo, found that inoculation of the yolk sac gave a higher percentage of infections with fewer deaths from trauma than when the inoculum was introduced into the allantois. However, Chaubaud (1939), inoculating on the allantois, carried *T. brucei* through fifteen passages. It appears from these references that the chick embryo has not been thoroughly investigated as a medium for cultivation of trypanosomes. The present investigation has as its primary objective the question of the cultivation of the trypanosomes serially in the developing embryo. Later developments suggested that the work be extended to include attempts to infect chickens with the newly developed cultures.

Certain difficulties were encountered as might be anticipated in the actual experimental studies, but with refinements in procedures it became possible to grow the trypanosomes in series, at will, in chick embryos. The primary cultures, as is commonplace in the experience of all workers whether dealing with viruses or bacteria, were the most difficult to obtain. However, it was rarely necessary to make more than one attempt in this direction once the conditions were standardized. By making small holes in the shell it was possible to obtain droplets of blood without apparent injury to the embryo. In this manner the multiplication of the trypanosomes could be observed, the morphology studied and the best time for transfer to new embryos noted. There seemed to be no limit to the number of serial passages in this medium. The morphology of the parasites grown in chick embryos as revealed by direct examination and Romanowsky stains seemed to be identical with the forms in the natural mammalian hosts. On this basis it would seem feasible to employ the cells cultured in chick embryos for the same type of investigation in which this medium has been so successful with viruses.

The question of infecting chickens with these three strains of trypanosomes has been subjected to experimentation on various occasions with negative results. In all instances the inoculum was infected mammalian blood. Several attempts were made in the present study to infect chickens, by injecting guinea pig blood, without success. However, in two instances occult or inapparent infections seemed to exist, and gave some encouragement for an extension of this work.

The present study made available organisms, from two sources, which conceivably might have developed some resistance to the defensive mechanisms of the chicken, (1) the fluids, rich in parasites, from infected embryos, and (2) the blood of chickens hatched from infected embryos in which the infection persisted. Suspensions of organisms from these sources were injected into a large number of three to seven day old chicks without the establishment of an infection.

Naturally the results were disappointing, but, on further scrutiny, appear

to offer an unusual opportunity for the investigation of natural immunity. The chick embryo, by the techniques developed, is an excellent medium for the multiplication of these trypanosomes and the infected embryos which continue to develop and hatch give rise to chickens with blood stream infections. On the one hand the infection disappears in most chickens within forty-eight hours, while those in which the infection persists are by no means normal and healthy since the yolk is incompletely absorbed, some deformities are present and death occurs within a week.

Fundamental knowledge leading to an understanding of immunity is woefully inadequate. In the present experimental work one observes embryos highly susceptible to infection. Within forty-eight hours this state is replaced by a solid natural immunity. Unquestionably an extension of the techniques described offer a means of gaining an insight into an understanding of this enigma.

SUMMARY AND CONCLUSIONS

T. equiperdum, *T. brucei* and *T. hippicum* were cultured serially on chick embryos with ease by the methods developed. The growth of the cultures was followed by removing blood samples through cuts made in the shell and subculturing was carried out on the basis of the findings. Embryos inoculated on the twelfth to fourteenth day continued to develop and more than fifty percent hatched. The infection either persisted in the chick causing its death on the fourth to the sixth day or disappeared within forty-eight hours after hatching. It was impossible to infect three to seven day old chicks by injecting them with suspensions of trypanosomes from guinea pigs, chick embryo cultures or blood of chicks hatched with heavy infection.

The morphology of the parasites in the chick embryo cultures or in the infected chicks was identical with that observed in the mammalian hosts.

The cultures of the trypanosomes in chick embryos are uniform and easy to duplicate and the same is true of the infection in white rats and white mice. As a result, two new tools are readily available for the determination of the trypanocidal action of chemotherapeutic agents. In addition, the luxuriant growth of the organisms in the chick embryo could be utilized to provide enormous numbers of parasites for such purposes as vaccines should such a need arise.

Attention should be directed to the fact that the data apply to only one strain of each of the three species of trypanosomes. Whether these data will be confirmed when these studies are extended to include other strains cannot be assured. However, the three strains are typical and of known origin.

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THE SUSCEPTIBILITY OF AFRICAN WILD ANIMALS TO YELLOW FEVER¹

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I. MONKEYS

INTRODUCTION

During recent years the staff of the Yellow Fever Research Institute in Entebbe, Uganda, has been engaged in a comprehensive study of various factors related to the epidemiology of yellow fever in central Africa. In the course of this study it has become evident (1-3) that in Africa, as in South America, jungle yellow fever occurs without necessarily involving man or the classic insect vector *Aedes aegypti*. The discovery of this fact required that attention be given to the insects and animals present in forested regions in order to learn whether they can and do enter into the epidemiological picture. In this paper are reported the results of studies of the susceptibility of various species of African wild animals to yellow fever.

Three factors determine whether a wild animal species is of significance in the epidemiology of yellow fever: (a) whether it is in nature attacked by the appropriate vector insects, (b) the duration of the period in which the virus is present in the blood and (c) the extent of multiplication of the virus in the host as reflected by the quantity of virus in the blood. Two general methods of approach are therefore available in determining the role of wild animals in natural cycles of infection: surveys to ascertain whether and with what frequency immunity to yellow fever occurs in various species, and tests of the ability of each to support the propagation and permit the circulation of virus in quantity sufficient to infect vector insects. The summation of information obtained by the 2 methods should permit an evaluation not only of the potential, but also of the actual, epidemiological importance of the species concerned.

Findlay, Stefanopoulo, Davey and Mahaffy (4), Findlay and MacCallum (5) and Van den Berghe (6) had already found immunity to yellow fever in primates from several regions of Africa. Our first comprehensive report on this subject (2) confirmed and extended their observations. More recently we have continued our surveys of immunity among the primates of east Africa and have extended the studies to include other orders (7). Among animals other than primates, we have found, in the course of a fairly comprehensive study, only 1 hyrax (*Procavia* sp. indet.²) and 1 mongoose (*Ichneumia* sp. indet.³)

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² This animal was one of 4 captured near El Obeid, in the Anglo-Egyptian Sudan, and supplied to us by Dr. E. S. Horgan, Director, Stack Memorial Laboratories, Khartoum.

³ Trapped in the compound of this Institute.

naturally immune. Other investigators (4) have likewise failed to find evidence of the importance of non-primates in the epidemiology of yellow fever. However, the number of species of African primates observed to be immune in nature is now large, and includes the following:

Investigators	Species ⁴	Country
Findlay, Stefanopoulo, Davey and Mahaffy (4)	<i>Anthropopithecus troglodytes</i> Linn. <i>Papio</i> sp. <i>Colobus badius</i> (ssp.nov.)	French Guinea Belgian Congo Gold Coast
Findlay and MacCallum (5)	<i>C. aethiops centralis</i> Neumann <i>Colobus vellerosus</i> Elliot <i>Cercopithecus</i> ? sp. <i>C. diana diana</i> <i>Procolobus badius badius</i> Pocock <i>Procolobus badius waldroni</i> Hayman	Sudan and Uganda Gold Coast, Sierra Leone Liberia Gold Coast Gold Coast Gold Coast
Van den Berghe (6)	<i>Colobus polykomos</i>	Belgian Congo
Haddow, Smithburn, Mahaffy and Bugher (2)	<i>Cercocebus albigena johnstoni</i> <i>Cercopithecus mitis stuhlmanni</i> <i>Cercopithecus mona denti</i> <i>Cercopithecus nictitans mpangae</i> <i>Colobus polykomos uellensis</i> <i>Papio doguera tessellatus</i>	Uganda Uganda Uganda Uganda Uganda Uganda
Haddow, Smithburn and Dick (7) ⁵	<i>Cercopithecus aethiops centralis</i> <i>Cercopithecus aethiops johnstoni</i> <i>Cercopithecus neglectus</i> <i>Colobus badius ellioti</i> <i>Colobus badius tephrosceles</i> <i>Colobus polykomos kikuyuensis</i> <i>Pan troglodytes schweinfurthii</i> <i>Galago crassicaudatus lasiotis</i>	Uganda Kenya Uganda Uganda Uganda Kenya Uganda Kenya

In our own experience with monkeys in Uganda it is only the species of which insufficient numbers of specimens have been tested, or those whose habitats are in regions where yellow fever has not occurred recently, which have not been found immune in nature. Since the only natural mode of transmission of yellow fever known is by the bite of insects, it is reasonable to assume from the immunity surveys that insect species capable of transmitting the disease do frequently attack monkeys under natural conditions. Furthermore, it has recently been determined by direct observation in Uganda (9) that 2 widely distributed species of monkeys are attacked in forest canopy by *Aedes* (*Stegomyia*) *africanus* Theo., a mosquito now known (3) to be a vector of yellow fever in central African forests.

⁴ When referring to the reports of other authors we quote the names used by them, but for our own work we employ the terminology adopted by G. M. Allen (8).

⁵ Immunity has also been observed in white-nosed guenons taken in various forests in central Uganda, but these are not here differentiated from those of the Semliki Forest (2).

However, the observation that a given species of animal acquires immunity to yellow fever in nature does not necessarily implicate that species in natural cycles of infection. If the animal circulates virus for only a very brief period, or in only small quantity, it may be involved only in a "dead end" infection, as pointed out by Waddell and Taylor (10) and may not serve in the perpetuation of the disease. The study of the possible rôle of animals in natural epidemiological cycles therefore resolves into the investigation of the extent of multiplication of virus in each and of the duration of the period in which it is present in the blood, or in studies of the ability of the species to maintain the virus in cyclic transmission (10).

In the days prior to the use of white mice in yellow fever investigations, tests of the reactions of various species of African monkeys to yellow fever virus were made, as follows:

Investigators	Species tested ⁶
Stokes, Bauer and Hudson (11)	A number of local species of African monkeys ⁷
Pettit, Stefanopoulo and Kolochine (12)	<i>Cercopithecus callitrichus</i> <i>Cercopithecus griseo-viridis</i> <i>Papio sphinx</i> <i>Cynocephalus hamadryas</i>
Bauer and Mahaffy (13)	<i>Cercocebus torquatus</i> <i>Cercopithecus mona</i> <i>Cercopithecus tantalus</i> <i>Erythrocebus patas</i>
Pettit and Aguessy (14)	<i>Pan satyrus</i>

The methods in use in the aforementioned studies showed that certain African monkeys exhibit circulating virus following inoculation or infection by mosquito bites, that they may, in consequence, be capable of infecting mosquitoes, and that they develop protective antibody as result of the infection. However, the limitations of the method precluded quantitative studies which would have shown the extent of virus multiplication and therefore have thrown light on the probable rôles of the animals in natural infections. The discovery by Theiler (15, 16) that white mice are susceptible to yellow fever virus made possible more informative experiments along this line. The following investigations on African monkeys, employing mice to determine the presence and quantity of circulating virus, were subsequently made.

Investigators	Species studied
Theiler and Hughes (17)	<i>Lasiopyga callitrichus</i>
Hughes (18)	<i>Cercopithecus aethiops centralis</i>

The *Lasiopyga callitrichus* studied by Theiler and Hughes (17) is probably identical with *Cercopithecus callitrichus*, studied by Pettit, Stefanopoulo and Kolochine (12). Moreover, *Cercopithecus* (*Lasiopyga*) *cellitrichus*, *C. griseo-*

⁶ The terminology used here is that employed by the authors referred to.

⁷ These tests were made in Lagos, Nigeria. All the monkeys were refractory to yellow fever virus.

viridis, *C. tantalus* and *C. aethiops centralis* are all members of the *C. aethiops* group of monkeys, so that the total number of species (or groups) tested for susceptibility is, strictly speaking, 7. These are: *Pan satyrus*, baboons of 2 varieties (families), *Cercopithecus mona*, *Erythrocebus patas*, *Cercocebus torquatus* and various subspecies of *C. aethiops*. Only the last named has been studied by quantitative methods.

The aforementioned studies have not shown any African primate to be as susceptible to African strains of yellow fever virus as the Indian monkey *Macaca mulatta*. In general, most of the species studied have survived and become immune, but some have been shown to circulate virus and to be capable of infecting mosquitoes. The present study embraces the application of appropriate techniques to the further investigation of 2 species of primates previously studied (13, 18) and of 10 species or subspecies which have not been studied previously. Although 1 genus, *Cercocebus*, is inadequately represented in this study by a single animal on which the experiment was none too satisfactory, conclusive results were obtained with all the other genera and species which are of sufficiently wide distribution to be of general importance, except *Colobus badius*. The latter, although it occurs in Uganda, has not been obtained alive for these tests. Studies on animals other than primates are considered separately (19, 20).

MATERIALS AND METHODS

Animals. A few of the animals employed were obtained by trapping, but most of them have been purchased, or were supplied to us by friends.⁸ The following species of monkeys were included in the studies:

Cercocebus albigena johnstoni (Lydekker)
Cercopithecus aethiops centralis Neumann
Cercopithecus lhoesti lhoesti P. L. Slater
Cercopithecus mitis kibonotensis Lönnberg
Cercopithecus mitis kolbi Neumann
Cercopithecus mitis stuhlmanni Matschie
Cercopithecus nictitans mpangae Matschie⁹
Erythrocebus patas pyrrhonotus (Hemprich and Ehrenberg)
Papio doguera tessellatus Elliot
Colobus polykomos kikuyuensis Lönnberg
Colobus polykomos uellensis Matschie
Pan troglodytes schweinfurthii Giglioli

Strains of Virus. Three strains of virus, each of them isolated in Africa, were used in the studies: the Asibi strain isolated in west Africa (11), a strain isolated from *Aedes simpsoni* in Uganda in 1942 and another isolated in Uganda

⁸ We wish to express our thanks to Dr. P. C. C. Garnham, then Specialist, Senior Parasitologist, Kenya Medical Service; to Mr. G. R. C. van Someren, Nairobi Municipal Council; to the Australian Zoological Expedition in East Africa; to Capt. C. R. S. Pitman, Game Warden, Uganda; and to Dr. James Hunter, District Medical Officer, Uganda Medical Services, for obtaining valued specimens for us.

⁹ Two of the monkeys so classified came from Bwamba County, Uganda, and the third from Entebbe. No attempt is here made to differentiate these, although Allen (8) does so.

in 1944 (1) from a mixed lot of *Aedes* mosquitoes. Each had been passed in the laboratory only in monkeys and was, in consequence, fully hepatotropic, the only exceptions being that one monkey received first mouse-brain passage Asibi virus and another was infected by the bites of mosquitoes. Also with these two exceptions, all monkeys were inoculated with desiccated monkey-serum virus especially rehydrated for this purpose. All inoculations of monkeys were made subcutaneously.

Doses of Virus. The inoculum for each animal was titrated by the intracerebral inoculation of groups of 12 mice^{10, 11} with serial tenfold dilutions of the virus. End points were determined by the method of Reed and Muench (21) and the end-point dilution was regarded as containing 1 LD₅₀ of virus per unit inoculum for mice (0.03 ml.) In some instances large doses of virus were intentionally used, but the inoculum for most animals was intended to be within the range which could, in nature, be transmitted by a small number of mosquitoes.

Tests for Circulating Virus. Daily tests were made for circulating virus in each inoculated animal. The animal was bled, serial dilutions of its serum were made in 10 per cent of non-immune monkey serum in physiological saline¹² and each dilution was tested by the intracerebral inoculation of 6 normal mice. It was intended to test a sufficient number of dilutions on each day to obtain an end point, and this was usually accomplished, but in a few instances when the peak titre occurred earlier or later than was expected complete end points were not obtained. The volume of inoculum for mice being 0.03 ml., the amount of virus in each ml. of serum of the monkey could be determined by multiplying the titre by 33.3.

Protection Tests. All protection tests were done by a sensitive method (22) which employs 1 per cent mouse-brain neurotropic virus inoculated intraperitoneally either into young adult mice, previously prepared by the intracerebral inoculation of 2 per cent starch, or into 11- to 14-day-old mice not inoculated with starch. A preliminary protection test was done on each monkey to determine its suitability for the experiment. Another test was done on each animal at the end of the experiment. In some instances, the residues of sera which had been collected for the tests for circulating virus were examined by the protection test to determine when antibody became demonstrable.

Observation of Animals. Rectal temperatures of monkeys were taken once on Sundays and holidays and twice daily on other days. Autopsies were done on all monkeys which died, and stained sections of their tissues were prepared and examined. Notice was taken of any intercurrent factors which might have influenced the course of the experiments.

Inoculated mice were examined daily in the forenoon, and records were made of the findings. Any which succumbed within 4 days (3 days in the protection tests, as the neurotropic virus sometimes causes death in 4 days) were regarded

¹⁰ Animals were anaesthetized with ether for this and other procedures.

¹¹ The mice used were descended from Swiss stock originally obtained from Carworth Farms, New York.

¹² Hereafter referred to as serum-saline.

as having died of extraneous causes. Protection test mice were observed for 12 days and all others for 21. Those which exhibited paralysis at the end of the period of observation were regarded as being specifically infected. All other mice which were alive at the end of the period of observation were regarded as not having been infected.

RESULTS

The results of the tests on 26 animals representing 6 genera and 12 species or subspecies of African monkeys are summarized in Table 1. Three of the species (*Cercocebus albigena johnstoni*, *Colobus polykomos uellensis* and *Pan troglodytes schweinfurthii*) are represented by tests on single specimens. Two of these, *Colobus polykomos uellensis* and *Pan troglodytes schweinfurthii* gave quite satisfactory results, but in the case of the single *Cercocebus*, virus was present in the blood for 2 days only, and then in the smallest quantity we have ever observed in any monkey. It is doubtful that this animal could have infected vector insects, but the results within other species have been so variable in the quantitative sense that there is question whether the reaction of this monkey can be regarded as typical for black mangabeys in general.

Mortality. Six of the animals died, either while the experiments were in progress or very shortly after the end of the usual 10-day period of observation. Intercurrent illnesses such as pyonephritis, gastric ulcer¹³ and pneumonia entered into the picture and were doubtless contributory causes of deaths. Only two of the animals which died failed to show some other contributory cause of death, and only these 2 are believed to have died of yellow fever. These were *C. aethiops centralis* M 278 and *Colobus polykomos kikuyuensis* M 546. The former will be discussed in the protocols which appear in a later section.

Colobus polykomos M 546 died on the 12th day after inoculation. Although its blood was negative on the day of death, yellow fever virus was recovered from the liver (Table 1). Histological examination revealed a moderate amount of fatty degeneration in the liver, occasional necrotic hepatic cells, some of them containing acidophilic intranuclear inclusions; and engorgement of Kupffer cells with the debris of necrotic liver cells. There was also a moderately severe nephritis, and lesions resembling those of yellow fever were found in the spleen. No contributory cause of death was found.

Thus, although 1 of 3 *C. aethiops centralis* and 1 of 3 *Colobus polykomos* succumbed as a result of the inoculations, the total mortality from yellow fever in the entire study was low. It seems probable that relatively few deaths from yellow fever occur in nature among the species investigated.

Febrile Reactions. Most of the monkeys showed definite rises in temperature above their normal values. *Colobus* 731 died 8 days after inoculation and was found to have an enormously dilated stomach which had displaced the liver downward into the pelvis. A normal temperature response was not, therefore, to be expected of this animal. Baboons M 265 and M 266 (Table 1) had no

¹³ This condition was found in *Erythrocebus patas* M 275. It has never before or since been seen here in any African monkey.

febrile reaction. Two other monkeys had such irregular temperatures that their febrile responses to the yellow fever virus were questionable. These were: *C. mitis stuhlmanni* 267 and *C. nictitans mpangae* 277. The observed temperature reactions in other monkeys were variable and, in general, not profound. Most of the animals had a significant rise in temperature starting 1 or 2 days after the appearance of virus in the blood, and persisting for 3 or more days. Not only did the first fever lag behind the first appearance of virus in the blood, but the peak of the febrile reaction was usually after the peak titre of virus in the circulation. Moreover, the fever persisted for 1 or more days after the virus had disappeared from the circulation.

Clinical Reactions. During the period of the febrile reactions it was usual for the animals to show some diminution in activity and some loss of interest in their food. The outward manifestations of illness were not profound, in most instances, and vomiting and icterus were not observed in any of the animals. Recovery was prompt, after the termination of fever, and apparently complete.

Circulating Virus. Each of the monkeys exhibited virus in its circulating blood for 2 or more days. The longest period of circulating virus was 9 days (*Colobus* M 546) and the average for all the animals was 4.27 days. The average duration of circulating virus in the 5 animals receiving very large doses was 3.0 days, whereas the average for all the others was 4.57 days. Furthermore, all the animals which received large doses had virus in their sera on the first day after inoculation, while all those receiving moderate or small doses failed to exhibit circulating virus during the first 1 to 5 days.

In the case of animals receiving very large doses it is not possible to state that the virus multiplied within them, as the peak titres in the circulation represented, in each instance, only a small fraction of the dose inoculated. (Those experiments have no parallel in nature, however, as it is probable that the great majority of infections in humans and animals are induced by the bite of a small number of mosquitoes, often 1).

All the monkeys receiving small or moderate doses exhibited virus in their sera for 2 or more days, and the peak titres were such as to indicate a marked increase over the amount introduced. There is little question that most of the monkeys receiving doses comparable to those which might be acquired naturally exhibited sufficient virus in the circulation, for a short period at least, to infect mosquitoes. Tables 2 to 8 show the titres of circulating virus by days in 7 animals and illustrate this point.

Immunity Acquired as a Result of the Infection. As stated above, protection tests were done on all the monkeys at the end of the experiments. All animals which lived 10 days or longer after inoculation were found to have developed protective antibody. In addition, the residues of sera collected daily for tests for circulating virus were, in some instances, examined by the yellow fever protection test to determine how early the antibody made its appearance.¹⁴ The latter

¹⁴ Sera collected in the first 10 days after inoculation were invariably inactivated by heating for 1 hour at 56° C. prior to use in the protection test in order to destroy any virus present.

TABLE 1
Condensed data from the tests of susceptibility to yellow fever in the 26 monkeys

ANIMAL NO.	SPECIES	INFECTED WITH		FEBRILE REACTION			CIRCULATION VIRUS			SERUM FIRST PROTECTIVE DAY	REMARKS
		Virus strain	Dose, mouse LD ₅₀	Elevated temp.* days	Maxi- mum °F.	Days of maximum	Days tested	Days present	Maximum, LD ₅₀ /ml. serum		
745†	<i>Cercopithecus albigena johnstoni</i>	Asibi	92	5 to 8	103.5	5	1 to 10	6 and 7	<33	10	
256	<i>Cercopithecus aethiops centralis</i>	Uganda A. simpsoni '42	10, 123,000	0 to 8	105.2	7	1 to 10	1 to 4	332	15†	
278	<i>Cercopithecus aethiops centralis</i>	Uganda A. Simpsoni '42	20	4, 5	104.2	4, 5	1 to 8	2 to 8	33,300,000†	8	Died 9th day
500	<i>Cercopithecus aethiops centralis</i>	Asibi, first mouse pass.	28,500,000	7 to 11	105.0	7	1 to 10	1 to 3	13,035	10†	
274	<i>Cercopithecus phoebe's phoebe's</i>	Uganda A. simpsoni '42	20	1, 2, 6, 8	103.4	1, 2, 6, 8	1 to 9	5 and 6	333	9	
486	<i>Cercopithecus phoebe's phoebe's</i>	Asibi	3,396	9, 10	105.0	9	1 to 10	2 to 5	2,297,700	6†	
506	<i>Cercopithecus mitis kibonolensis</i>	Asibi	460	4 to 9	104.0	8	1 to 10	3 to 5	76,550	6	
567	<i>Cercopithecus mitis kibonolensis</i>	Asibi	460	4 to 10	105.2	8	1 to 10	3 to 5	765,900	6	
752	<i>Cercopithecus mitis kibonolensis</i>	Asibi	92	4, 5, 8	104.6	8	1 to 10	2 to 7	1,911,000	7	Died 10th day§
751	<i>Cercopithecus mitis kolbi</i>	Asibi	92	4, 5, 0, 10	105.1	9	1 to 10	3 to 7	58,275,000	8	
754	<i>Cercopithecus mitis kolbi</i>	Uganda Aedes '44	24	5 to 8, 10	104.0	7	1 to 10	3 to 7	113,220	8	
254	<i>Cercopithecus mitis stuhlmanni</i>	Uganda A. simpsoni '42	16, 123,000	8 to 11	105.0	10, 11	1 to 10	1 to 3	16,983	15†	
267	<i>Cercopithecus mitis stuhlmanni</i>	Uganda A. simpsoni '42	92		—	—	1 to 10	2 to 5	592,740	10†	
859	<i>Cercopithecus mitis stuhlmanni</i>	Uganda Aedes '44	120	5, 8	104.2	8	1 to 10	2 to 5	83,916	7	
257	<i>Cercopithecus nictitans mpangae</i>	Uganda A. simpsoni '42	14,300,000	7 to 9	104.8	9	1 to 10	1 and 2	1,056	15†	
277	<i>Cercopithecus nictitans mpangae</i>	Uganda A. simpsoni '42	20		—	—	1 to 9	4 to 9	105,560†	Neg. 9th day	Died 10th day§
481	<i>Cercopithecus nictitans mpangae</i>	Asibi	3,396	11	104.2	11	1 to 10	2 to 5	2,464	6†	
275	<i>Erythrocebus palus pyrrhonotus</i>	Uganda A. simpsoni '42	20	6 to 9	105.0	8	1 to 8	3 to 6	839,100	Incon. 7th day	Died 8th day§
564	<i>Erythrocebus palus pyrrhonotus</i>	Asibi	460	4, 8	103.0	8	1 to 10	3 to 7	33,300	8	
255	<i>Papio doguera tessellatus</i>	Uganda A. simpsoni '42	10, 123,000	5	103.8	5	1 to 10	1 to 3	10,556	15†	
265	<i>Papio doguera tessellatus</i>	Uganda A. simpsoni '42	92	No	—	—	1 to 10	3 to 6	33,300	10†	
266	<i>Papio doguera tessellatus</i>	Uganda A. simpsoni '42	92	No	—	—	1 to 10	3 to 5	592,740	10†	
546	<i>Colobus polykomos kikuyuensis</i>	Asibi	269	8, 10	103.4	8, 10	1 to 10, 12	2 to 10	66,600,000	12†	Died 12th day Liver positive, blood negative for virus

549	<i>Colobus polykomos kikuyuensis</i>	Asibi	Mosquitoes (2 probed, 4 gorged)	5	103.0	5	1 to 14	3 to 8	131,530	8	
731	<i>Colobus polykomos uellensis</i>	Asibi		5.6	—	—	1 to 7	2 to 7	3,330,000	—	Died 8th day. Anatomical defect contributory.
908	<i>Pan troglodytes schweinfurthii</i>	Uganda <i>Aedes</i> '44	400	5, 8	101.0	5, 8	1 to 10	3 to 6	145,854	8	

* Significant elevation above normal for each animal, the normal varying with the species.

† These are serial numbers used in this laboratory over a period of many years.

‡ No earlier protection tests were done.

+ Indicates end point exceeds figure shown but is unknown because deaths were caused by highest dilution tested.

§ Result complicated by intercurrent illness.

|| Doubtful reaction on account of variability unassociated with inoculation.

‡ Monkey 277 came from Entebbe and the other 2 of this species from Bwamba County. No attempt is made to differentiate these subspecifically. See footnote 5, p. 390

study yielded some quite interesting results. It was found that in some monkeys the production of antibody proceeded so rapidly that the serum was non-protective one day and fully protective the next (M 752, Table 3). In other cases the rise in antibody titre was more gradual, and successive daily specimens of sera gave first negative, then inconclusive and finally positive (protective) reactions, as shown by M 859, Table 4, and M 908, Table 8. There can be little doubt that such inconclusive reactions, preceded by negative and followed by protective sera, represent partial protection, and that the amount of antibody present is inadequate to protect fully against the quantity of virus used in the test.

Antibody in sufficient concentration to give the serum complete protective power in the routine protection test was occasionally demonstrable (Tables 2 and 3) at a time when virus was still present in the blood. (This observation is, however, not original, as similar findings have been reported by Berry and Kitchen (23) and others). In other cases the development of full protective power was delayed until 1 or 2 days after the disappearance of virus from the circulation.

Protocols. The condensed data presented in table 1 do not reveal in detail the characteristics of the responses of individual animals. Although it is not possible to present here the complete protocols of the studies on all the monkeys, a few of the experiments will be given more fully.

1. A juvenile grey monkey, *Cercopithecus aethiops centralis* M 278, was inoculated subcutaneously with 1.0 ml. of 1 in 1,000,000 dilution of rehydrated yellow fever virus of the strain isolated in 1942 from *Aedes simpsoni* (1). The titre of this virus was such that the inoculum for M 278 represented 20 LD₅₀. The animal showed no sign of illness until the fourth day, when it first had fever. It thereafter exhibited anorexia, its coat became rough and its condition became progressively worse. It was found dead on the morning of the 9th day.

At autopsy the liver was found to be yellowish-pink in color and of normal size. It did not appear to be greasy. There were tiny flecks of black, altered blood in the stomach. No other noteworthy gross abnormalities were found. Sections of liver exhibited extensive fatty degeneration, numerous small hemorrhages, moderate necrosis of hepatic cells and moderate numbers of typical acidophilic intranuclear inclusions. The kidneys showed moderately severe glomerulo-tubular nephritis with some interstitial infiltration.

Table 2 shows the temperature reaction of M 278, the results of tests for circulating virus and the protection-test data. The high levels of circulating virus are noteworthy, as is also the fact that antibody was demonstrable on the 8th day when a considerable amount of virus was still present in the blood. A specimen of liver taken from M 278 at autopsy was tested for virus, with positive result, but the indications were that the amount of virus present was small.

The results obtained with M 278 and the other animals of this species are in agreement with the findings of Hughes (18) in studies on *C. aethiops centralis*.

2. M 752, a subadult male *C. mitis kibonotensis*, was inoculated with 1.0 ml. of 1 in 100,000 dilution of rehydrated Asibi virus. The titre of the inoculum

(276,000) was such that the dose for the monkey was 92 mouse-intracerebral LD₅₀. The animal showed elevated temperatures on the 4th, 5th and 8th days

TABLE 2

The reaction of C. aethiops centralis M 278 to inoculation with a small dose of the strain of virus isolated in 1942 from Aedes simpsoni

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO* OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			
	103.0			1/6
1	103.6	0	0	—
2	103.6	<1	<33	—
3	103.8	395	13,153	—
4	→104.2	100,000	3,300,000	—
5	→104.2	1,000,000+	33,300,000+	—
6	103.6	100,000+	3,300,000+	—
7	103.8	3,170+	105,560+	4/7, 6/8, 5/8
8	103.0	317+	10,556+	7/8

Arrows show days of febrile reaction.

* Numerator shows number of mice surviving, the denominator the number inoculated.

+ Indicates titre exceeded figure shown but was not determined because insufficient dilutions were tested.

— Indicates no test made.

TABLE 3

The reactions of C. mitis kibonotensis M 752 to inoculation with a small dose of the Asibi strain of virus

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			
	103.2			0/8
1	102.6	0	0	—
2	102.8	Tr*	<33	—
3	103.2	230	7,659	—
4	→104.0	57,400	1,911,420	—
5	→103.6	3,170	105,561	0/8
6	101.4	23	767	0/8
7	101.2	1.45	48	6/6
8	→104.6	0	0	8/8
9	103.3	0	0	—
10	102.8	0	0	8/8

* Tr indicates presence of virus, but in quantity sufficient to cause death of no more than 2 of 6 mice receiving undiluted serum.

Other legends as in table 2.

and ate poorly from the 5th day onward. On the 8th day it became dyspnoeic and showed signs of pneumonia. From the 11th day onward its temperature

was subnormal and it died on the 16th day. Its temperature records, the results of daily titrations of its serum for virus and the protection-test data are presented in Table 3.

Post-mortem examination revealed evidence of lesions resulting from the inoculation but showed that death was not caused by yellow fever virus. There was extensive fatty degeneration in the liver, but no acidophilic necrotic cells or inclusion bodies were found. There was a well-organized hemorrhage 1.5 cm. in diameter in the gastric mucosa, obviously of several days standing. Death was caused, in all probability, by pneumonia in the stage of gray hepatization, which involved the left lung. There was also an intense glomerulo-tubular nephritis, probably not caused by yellow fever virus.

The titre of virus in the blood of M 752 was certainly adequate to infect mosquitoes (see below) on the 3rd, 4th and 5th days if not at other times. Also noteworthy is the rapid production of antibody so that the serum was fully protective by the 7th day, when virus was still present in the blood.

3. M 859, a subadult female *C. mitis stuhlmanni* was inoculated subcutaneously with 1.0 ml. of 1 in 200,000 dilution of rehydrated yellow fever virus, of the strain isolated from *Aedes* mosquitoes in 1944. The titre of this virus was such (725,000) that the inoculum represented 120 mouse-intracerebral LD₅₀. The temperature reaction and the results of tests for circulating virus and of serial daily protection tests are shown in Table 4.

M 859 became slightly less active than normal during the period of fever and showed less interest in food than usual. It did not appear very ill and its recovery was prompt and complete after the fever subsided. The temperature of 103.0° which this animal showed on the 6th day has to be regarded as being within the normal range, but the temperatures of 104.0° and 104.2° on the 5th and 8th days are definitely high. This therefore represents a saddleback type of curve, which was not uncommon in these experiments.

It will be noted (Table 4) that M 859 had fairly high levels of circulating virus on the 3rd to 5th days, that some evidence of antibody was present on the 6th day and that the serum was fully protective on the 7th day.

4. A subadult white-nosed guenon (more commonly called locally the red-tail monkey), *C. nictitans mpangae*¹⁵ M 277, was inoculated subcutaneously with the same dose (20 LD₅₀) of the suspension of virus used to inoculate M 278. This animal's temperature was elevated prior to inoculation and remained so thereafter; its febrile response to the inoculation, if any, could not be differentiated. It did not appear ill at the time of inoculation but shortly thereafter developed anorexia and became progressively weaker. It was found dead on the morning of the 10th day. Histological examination revealed moderate fatty degeneration in the liver and necrosis of occasional hepatic cells, but no extensive acidophilic necrosis and no inclusions. The spleen, kidneys and adrenals showed evidence of a septic condition; it therefore appeared that death was not due to yellow fever.

¹⁵ See footnote Table 1 regarding this monkey.

Results of tests for circulating virus and of protection tests on M 277 are shown in Table 5.

A portion of liver removed at autopsy from M 277 was tested for yellow fever

TABLE 4

The reaction of C. mitis stuhlmanni M 859 to inoculation with a small dose of the strain of yellow fever virus isolated in 1944 from Aedes mosquitoes

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			
	103.0			0/8
1	103.0	0	0	—
2	103.6	<1	<33	—
3	102.8	178	5,927	—
4	102.6	2,300	76,590	—
5	→104.0	2,520	83,916	0/8
6	103.0	0	0	3/8
7	103.4	0	0	7/8
8	→104.2	0	0	8/8
9	103.6	0	0	8/8
10	102.6	0	0	7/8

Legends as in table 2.

TABLE 5

The reaction of C. nictitans mpangae M 277 to inoculation with a small dose of the pantropic yellow fever virus isolated in 1942 from Aedes simpsoni*

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			
	104.6			1/6
1	105.4	0	0	—
2	106.0	0	0	—
3	106.0	0	0	—
4	106.6	1	33	—
5	105.8	100	3,300	—
6	104.6	740	24,642	—
7	106.0	3,170+	105,560+	1/8
8	106.2	317+	10,556+	1/8
9	105.2	25	832	0/7

* See footnote ¶, Table 1.

virus, with positive result. It is also noteworthy that the animal exhibited virus in its blood for 6 days (table 5), in relatively high concentration for 3 of these, and that demonstrable antibody was not present in its serum on the 9th day.

5. A juvenile female baboon, *Papio doguera tessellatus* M 266, was inoculated

subcutaneously with 92 LD₅₀ of the strain of virus isolated in 1942 from *Aedes simpsoni*. The animal remained apparently quite well throughout the experiment and had no febrile reaction to the inoculation. The daily maximal temperatures, the results of tests for circulating virus and the outcome of protection tests on its preinoculation and "convalescent" sera are shown in Table 6.

This animal had an unusually short period of circulating virus, considering the relatively high level reached on the 4th day. Its complete lack of objective clinical reaction is probably representative of what occurs in very mild or sub-clinical cases in man. Another baboon inoculated at the same time with the same dose of virus (M 265, Table 1) gave a very similar response, exhibiting circulating virus for one day longer but failing to reach quite such a high maximum level.

TABLE 6

The reaction of P. doguera tessellatus M 266 to the inoculation of a small dose of the strain of yellow fever virus isolated in 1942 from A. simpsoni

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			0/6
1	102.2	0	0	—
2	103.2	0	0	—
3	101.6	23	766	—
4	101.4	17,800	592,740	—
5	102.8	74	2,464	—
6	102.2	0	0	—
7	102.0	0	0	—
8	102.4	0	0	—
9	102.0	0	0	—
10	101.8	0	0	—
	102.2	0	0	5/5

6. M 549, an adult male black-and-white colobus (*Colobus polykomos kilikuyensis*) was infected by allowing it to be bitten by *Aedes aegypti* fed 14 days previously on a rhesus monkey inoculated 4 days earlier with Asibi virus. Four mosquitoes gorged on the colobus and 2 others inserted but got no blood. Immediately after exposure to *Colobus* M 549 these 6 mosquitoes were triturated in 1.0 ml. of 10 per cent serum-saline. The suspension was filtered through a Seitz EK pad and inoculated intracerebrally into 6 mice, all of which died between the 10th and 12th days, showing that the mosquitoes contained virus.

Colobus M 549 had a mild febrile reaction on the 5th day only. It survived the experiment without appearing definitely ill at any time. This animal was used for tests to determine what level of circulating virus will give rise to enduring infection in *Aedes aegypti* (see appropriate section of this report).

The daily temperatures, results of titrations of serum and the protection-test data on *Colobus* M 549 are shown in Table 7. It is to be noted that the animal had virus in its serum for 6 days and that the quantity present was quite high

on the 4th to 6th days. Demonstrable antibody was present on the 8th day, before the virus had disappeared from the blood.

7. M 908, a juvenile male *Pan troglodytes schweinfurthii*, was inoculated subcutaneously with 1.0 ml. of 1 in 200,000 dilution of rehydrated yellow fever virus, strain isolated from *Aedes* mosquitoes in 1944. The titre of this virus (a different ampoule of the same batch used for inoculation of M 859) was 2,400,000, so that the dose for the chimpanzee represented 400 mouse-intracerebral LD₅₀.

The temperature curve of chimpanzee M 908 was fairly stable and the levels of 101.0° reached on the 5th and 8th days (Table 8) may be regarded as mild febrile responses. During this interval the animal was somewhat listless and ate less than normal. By the 10th day, however, it was again quite active and took food well. Virus was present in its blood from the 3rd to the 6th day, in good

TABLE 7

The reaction of Colobus polykomos kikuyuensis M 549 to infection by the bites of Aedes aegypti with the Asibi strain of yellow fever virus

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			
	103.0			0/10
1	103.0	0	0	—
2	101.8	0	0	—
3	103.2	<1	<33	—
4	101.4	395	13,153	—
5	→103.6	3,950	131,530	—
6	103.0	230	7,659	0/10
7	102.8	4	144	8/10
8	102.2	1	33	10/10
9	100.4	0	0	10/10
10	102.8	0	0	—

quantity on the 4th and 5th days. There was evidence of the presence of some antibody in its serum on the 7th day, and the 8th day serum was fully protective, as shown in Table 8.

THE AMOUNT OF CIRCULATING VIRUS REQUIRED TO INFECT MOSQUITOES

The Quantity of Blood Taken by Aedes aegypti. The significance of low levels of virus in the circulating blood of an animal or man can not be evaluated unless the amount of virus required to infect mosquitoes is known. The latter could, of course, be determined fairly accurately by comprehensive direct experiments. Such are, in fact, in progress here at present. Indirect evidence was obtainable by a simpler method, namely, by determining the quantity of blood taken by vector mosquitoes at a single feeding. The following experiment was done to obtain this information.

Forty-six freshly emerged female *Aedes aegypti* were given food and water the

first day, water only the second day and neither food nor water on the third day. On the afternoon of the third day, they were placed in 3 tubes, which were then sealed and carefully weighed on an accurate "Chainomatic" balance. The mosquitoes were then offered a guinea pig. Those which fed were separated from those which did not, and the former were weighed as promptly as possible after feeding. The weights of the tubes were then determined and from the data collected the weight of starved and gorged mosquitoes was calculated. The results were as follows:

	Gm.
Weight of 46 starved <i>A. aegypti</i>	0.0874
Mean weight of starved <i>A. aegypti</i>	0.0019
Weight of 38 gorged <i>A. aegypti</i>	0.1690
Mean weight of gorged <i>A. aegypti</i>	0.00444
Mean increase in weight.....	0.00254

The average amount taken was thus 133 per cent of the body weight of each mosquito and was equivalent to approximately 1/400 ml. of blood. Therefore, if an infected individual had just 400 units of virus per ml. of blood, *Aedes*

TABLE 8

The reaction of Pan troglodytes schweinfurthii M 908 to inoculation with a small dose of the strain of virus isolated in 1944 from Aedes mosquitoes

DAYS AFTER INOCULATION	MAXIMUM TEMPERATURE	VIRUS IN SERUM		PROTECTION RATIO OF SERUM
		Titre	LD ₅₀ per ml.	
Preinoculation	°F.			0/6
1	100.2	0	0	—
2	100.6	0	0	—
3	100.0	4	133	—
4	99.8	100	3,330	0/10
5	→101.0	4,380	145,854	0/9
6	100.2	7	233	1/10
7	100.4	0	0	6/10
8	→101.0	0	0	10/10
9	100.8	0	0	9/10
10	99.0	0	0	9/10

aegypti which attacked it would take up, on the average, only 1 particle of virus. It is not known whether a single virus particle, or even a larger, but still small, number can give rise to enduring infection in a mosquito, but it is certain that any level of circulating virus below 400 units per ml. would be unlikely to do so regularly. It seems most probable, on the other hand, that any mosquito which attacked an individual having 4,000 or more units of virus per ml. of serum would become infected. It is noteworthy that every species of monkey included in this study exhibited this or a higher level of circulating virus, with the single exception of the black mangabey, *Cercocebus albigena johnstoni*, of which species the single example in this study may not be representative.

Mosquitoes Infected on Colobus M 549. Laboratory-reared *Aedes aegypti* females 1 to 5 days old were offered *Colobus M 549* daily from the 3rd to 12th day after inoculation of the monkey. The number of mosquitoes used each day varied from 15 to 30, of which 12 to 25 fed. Of the total of 209 mosquitoes used, 179 (85.6 per cent) fed. Four mosquitoes of the lot fed on any given day were triturated in a mortar with 1.0 ml. of serum-saline and the supernatant fluid, after centrifugation, was passed through a Seitz EK pad previously washed with serum-saline. The filtrate was tested for virus by the intracerebral inoculation of a group of 6 mice.

Mosquitoes were kept separate by lots in Barraud cages in a controlled temperature cabinet at 30°C. At intervals of 24 to 44 days after their infecting feeds, tests for virus were made on individual mosquitoes or on pooled insects, either the individual or the pooled mosquitoes being triturated in 1.0 ml. of serum-saline as above. The 5th- and 6th-day lots of mosquitoes were also allowed to bite normal rhesus monkeys at intervals of 14 to 44 days after their infecting feeds. Results of the tests for virus immediately after the infecting feeds and after 24 to 44 days incubation are shown for the 3rd to 9th day lots of mosquitoes in Table 9.

From the data presented in Table 9 it will be seen that the mosquitoes which bit *Colobus M 549* on the 4th, 5th and 6th days after its inoculation acquired virus from the monkey as shown by tests of mosquitoes made immediately after their blood meals. Mosquitoes fed on other days, when the levels of virus were lower in the monkey, failed to show virus in tests made immediately after their feedings. It is therefore obvious that mosquitoes can not become infected when the level of virus in the blood of the host is below a certain value, somewhere between 7,659 and 144 LD₅₀ per ml.

The tests for virus after incubation of the mosquitoes yielded unexpected results for which there is no obvious explanation. Of 33 individual mosquitoes of lots shown by the immediate tests to have acquired infection, the persistence of virus was demonstrated in only 2. The reason for the failure of the mosquitoes to retain their infection is unknown, but the fact of this failure is further emphasized by the unsuccessful transmission experiments which were done with some of them. The lots fed on *Colobus M 549* on the 5th and 6th days (both shown to contain virus immediately after the feedings) were allowed to bite a total of 5 normal rhesus monkeys after incubation periods of 14 to 44 days. None of the 5 monkeys became infected, although they were bitten by 3 to 9 mosquitoes each. The *A. aegypti* which fed on *Colobus M 549* on the 4th day were not allowed to bite non-immune monkeys, and the single mosquito of the 5th-day lot which was shown to have retained the virus for 28 days did not bite a monkey. Therefore, none of the rhesus was bitten by mosquitoes which were proved to have retained the virus. Since this is the case, the failure of transmission is understandable, but the failure of the mosquitoes to retain the virus is not. Ross and Lumsden (24) have, moreover, observed the failure of *Aedes aegypti* which retained virus to transmit the infection. Nevertheless, successful transmissions with this and other species of mosquitoes have been carried out here (*Colobus M 549* was itself infected by the bites of the same

laboratory stock of *A. aegypti*) and it is possible that some unknown condition of these experiments was responsible for the failures.

Further studies on the levels of circulating virus required to infect vector insects will be reported later (24).

DISCUSSION

Certain general phenomena of the reactions of wild African monkeys to inoculation with pantropic yellow fever virus are demonstrated by the data presented. These are:

1. The larger the dose of virus inoculated, the earlier the agent appears in the serum. It will be seen from table 1 that only those animals which received very large doses exhibited circulating virus on the first day after inoculation.

TABLE 9

Tests for yellow fever virus in Aedes aegypti immediately and at various intervals after blood meals of differing virus content

SOURCE ANIMAL, <i>Colobus</i> M 549		TESTS FOR VIRUS IN <i>Aedes aegypti</i> FED ON <i>Colobus</i> M 549				
Day of infection	LD ₅₀ virus per ml. serum	Immediately after feeding		Days	After incubation	
		No. mosquitoes	Mortality ratio in mice*		No. tested	No. positive
3	33	4†	0/6	25	10†	0
4	13,153	4†	2/6	24	15‡	1 (P) X
5	131,530	4†	6/6	28	5‡	1 (P) X
				44	3‡	0
6	7,659	4†	5/6	42	10‡	0
7	144	4†	0/6	41	10†	0
8	33	4†	0/6	40	8†	0
9	0	4†	0/6	39	6†	0

* In these mortality ratios the numerators represent the numbers of mice dying and the denominators the numbers inoculated.

† Pooled and suspended in 1.0 ml. of diluent.

‡ Each mosquito suspended in 1.0 ml. of diluent and tested separately.

(P) Indicates that identity of virus recovered from these mosquitoes was proved by protection tests.

X Indicates that the mosquito shown to contain virus did not bite a normal monkey.

2. The larger the dose inoculated, the lower is the peak titre of circulating virus and the earlier the disappearance of the virus from the blood. The reactions of the 3 *C. nictitans* (table 1) are illustrative of this point.

3. The inoculation of a small dose of virus (within the range transmissible by the bites of a few mosquitoes) is followed by a true period of intrinsic incubation, during which the animal remains afebrile and exhibits no circulating virus until the 2nd to 5th day.

4. The introduction of a small dose of virus is followed by a longer period of circulating virus and a higher peak titre than is the case following larger doses.

5. In general, the febrile reaction does not appear until about the time the

peak titre of virus is reached in the blood. The fever usually persists for a short time after the disappearance of virus from the circulation. These facts seem to indicate that the febrile response is not a reaction to the presence of the virus *per se* in the tissues, but that it may be a reaction to the destruction of tissue by the virus.

While the studies reported here indicate that all the species of monkeys investigated (with the possible exception of the black mangabey) are sufficiently susceptible to the virus to be natural hosts of the disease, it does not necessarily follow either that they are of equal epidemiological importance, or even that they all *do* participate in natural cycles of infection. For example, *Cercopithecus lhoesti lhoesti* is a species which probably does not become infected often in nature owing to the fact that its natural habitat is mountain forest above the altitude at which yellow fever commonly occurs; this species does, on occasion, invade lowland forest in search of food, but any individuals which then became infected would probably not give rise to further infections among their fellows, owing to the absence of appropriate vectors in their normal habitat. *Erythrocebus patas* probably does not participate frequently in natural cycles of infection as it is not a resident of forest, but of open grassy savannah. On the other hand, several of the species studied here are normal inhabitants of the lowland forests of central Africa and are doubtless very commonly involved in natural cycles of infection, a fact emphasized not only by these studies but also by immunity surveys. These include *Cercopithecus aethiops*, *Cercopithecus nictitans*, *Colobus polykomos* and *Papio doguera*. *C. aethiops* and *C. nictitans* more commonly raid the plantations of man than do the others and are, therefore, the more likely links between the cycle of infection in forest and the human population. The other species, and perhaps additional ones not studied here, are probably of considerable importance in maintaining the disease within forest but have lesser rôles in bringing the infection to man. It seems unlikely that chimpanzees (although highly susceptible) and gorillas are of great importance, owing to their relative scarcity.

SUMMARY

Quantitative studies were made of the susceptibility to yellow fever of 12 species of African monkeys. All were found to be susceptible in the sense that they exhibited virus in the circulation, gave evidence of the multiplication of virus in their tissues and developed humoral antibody during convalescence. Every species save one (represented by a single animal) exhibited sufficient virus in the circulating blood to be capable, in all probability, of infecting mosquitoes.

The probable rôles of some of the species in natural cycles of infection are discussed.

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II. RODENTS, BUSH PIG, HYRAX AND LEOPARD

K. C. SMITHBURN AND A. J. HADDOW

A study was made of the susceptibility to yellow fever and possible epidemiological rôle in natural cycles of infection of the hyrax (*Procavia* sp. indet.), the Uganda red hog (*Potamochoerus porcus intermedius* Lönnberg), the leopard (*Felis pardus iturensis* (J. A. Allen)) and 7 species of rodents: *Lemniscomys striatus* (L.), *Arvicanthus abyssinicus* (Rüppell), *Cricetomys gambianus* Waterhouse, *Dendromus messorius* Thomas, *Claviglis murinus* Desmarest, *Mastomys coucha* (A. Smith) and *Thamnomys dryas* Thomas.

MATERIALS AND METHODS

The general procedures employed in the experiments were set forth in the previous section and need not be repeated here. However, certain departures from our standard methods were necessitated in the present study by the size of some of the rodents. The methods used in these instances will be described herewith.

Animals. The one hyrax available for study was captured in the vicinity of El Obeid, in the Anglo-Egyptian Sudan, and supplied to us by Dr. E. S. Horgan of the Stack Memorial Laboratories, Khartoum. The Uganda red hog, the leopard and all of the rodents were captured in Bwamba County, in western Uganda.

Tests for Circulating Virus. If the available number of animals of a species and the size of the individuals were both small, the usual daily tests for circulating virus could not be done. In the case of very small rodents of some species the only criterion of susceptibility was a final test for acquired immunity. In the case of somewhat larger animals, if enough were available to insure that some would undergo their natural reactions without influence from technical procedures, individuals were bled on alternate days and their sera tested for virus. In this way, the reactions of 2 to 4 animals could be taken as representative of the group. Animals of sufficient size to permit obtaining daily samples of serum were tested for circulating virus each day.

Protection Tests. The small size of some of the rodents precluded the usual protection tests. In such instances pooling of the blood of 2 to 4 animals of the same species was sometimes resorted to. However, during the course of the experiments another method was devised which made possible routine protection tests on animals whose small size prohibited the procurement of sufficient serum for the usual test and which obviated the necessity for pooling sera of different animals. This involved the use of extracts of liver in lieu of serum. The animal was sacrificed with chloroform and a portion of liver (devoid of gall-bladder) was removed aseptically. This was ground in a mortar with sterile powdered pyrex glass as abrasive and suspended in sufficient physiological saline to make a 10 or 20 per cent suspension. The latter was spun at approximately 2500 r.p.m. in the angle centrifuge, after which the supernate was removed, care being taken to avoid the fatty topmost layer. This supernate (sometimes but not invariably Seitz-filtered) was used instead of serum. Appropriate control tests,

employing liver extracts of known normal and immune animals, demonstrated that the extracts of liver gave reactions in the protection tests which faithfully recorded the presence or absence of immune bodies. Utilization of this method in some of the later studies on rodents made possible some protection tests which could not have been done had dependence been placed on obtaining sufficient serum for examination.

Inoculations. All inocula were titrated in mice as in the studies on monkeys (1), so that the quantity of virus given each animal was known. Most of the animals were inoculated subcutaneously, but rodents of certain species were inoculated intracerebrally in order to determine whether the neurotropic attribute of yellow fever virus was effective in them.

PROCEDURES AND RESULTS

Experiment I. Thirty-three adult *Lemniscomys striatus* and 37 adult *Arvicanthus abyssinicus* were each inoculated subcutaneously with 0.5 ml. of 1 in 50 dilution of rehydrated pantropic yellow fever virus of the strain isolated in 1942 from *Aedes simpsoni* (2). The titre of the inoculum was such that each animal received 205,666 LD₅₀ of virus.

Successive pairs of each species were bled on each of 16 consecutive days, and their sera were tested for virus by the intracerebral inoculation of groups of 6 mice. None of the mice died of yellow fever encephalitis, so that no virus was demonstrated in any of the 64 animals tested.

Blood specimens of 31 *Arvicanthus abyssinicus*, taken 22 to 25 days after the inoculations, were examined by the yellow fever protection test. Thirty gave non-protective reactions and only 1 was protective.

Difficulty was encountered in obtaining sufficient serum from the *Lemniscomys striatus* for individual protection tests, and in some instances it was necessary to resort to pooling. Blood samples from 18 animals, taken 29 or 37 days after their inoculations with yellow fever virus, were examined as 11 specimens, with results as follows:

- 7 single specimens taken on the 29th day, all non-protective.
- 1 pool from 2 animals bled on the 37th day, non-protective.
- 1 pool from 2 animals bled on the 37th day, protective.
- 1 pool from 3 animals bled on the 29th day, protective.
- 1 pool from 4 animals bled on the 37th day, protective.

Thus a minimum of 3, and a maximum of 9, of the 18 animals exhibited protective antibody in their sera 29 to 37 days after inoculation.

Fifteen immature *Arvicanthus abyssinicus* born in captivity in Entebbe were each inoculated intracerebrally with 0.03 ml. of the 1 in 50 dilution of the same virus suspension used for the preceding animals, the dose in this instance being 12,340 mouse-intracerebral LD₅₀. All of these animals remained well, and they were discarded on the 25th day after inoculation.

The results obtained in this experiment show a very low degree of susceptibility to yellow fever virus in these 2 species of rodents and indicate that they probably have no importance in the epidemiology of the disease.

Experiment 2. Six non-immune giant rats (*Cricetomys gambianus*) were each inoculated subcutaneously with 132,200 mouse-intracerebral LD₅₀ of pantropic yellow fever virus, of the strain isolated in 1942 from *Aedes simpsoni* (2). These animals were very fierce and strong and had to be given chloroform anesthesia for all procedures. Two succumbed to the anesthetic 24 hours after inoculation and 3 others died, either from the anesthetic or from technical procedures, 48 hours after inoculation. The 6th died on the 7th day of hemopericardium resulting from cardiac puncture. Neither of the 2 tested on the first day exhibited circulating virus. Only 1 animal (the same each day) was tested thereafter. It exhibited virus in its serum on the second and third days only, the titres being, respectively, less than 1, and 56. This animal had neutralizing antibody against yellow fever virus in its serum on the 6th day (protection ratio of serum: 5/6).

No further experiments have been done with *Cricetomys gambianus*, but the short period of circulating virus and the relatively low peak titre in the 1 animal giving a good test indicate that this species is probably of little importance in natural cycles of yellow fever. Further evidence on this point is the fact that we have found no immunes among a considerable number of this species captured in Bwamba County, Uganda, where yellow fever is endemic.

Four tree mice (*Dendromus messorius*) were each inoculated intracerebrally with 3,970 LD₅₀ of the same strain of yellow fever virus as was used in the preceding tests. The small size of the animals precluded preinoculation protection tests. One of the animals died immediately and one on the 7th day; a sick one was sacrificed for passage on the 8th day and the virus was recovered from its brain but only in low titre. The fourth animal survived; a protection test on its serum, taken 14 days after inoculation, gave a positive (protective) result. This experiment can not be regarded as either conclusive or exhaustive, but the results seem to indicate that *Dendromus messorius* is not highly susceptible to yellow fever virus inoculated intracerebrally.

Four *Claviglis murinus* (tree dormice) were inoculated intracerebrally with 3,970 LD₅₀ of the pantropic virus isolated from *Aedes simpsoni*. One died immediately; moribund animals were sacrificed for passage on the 8th and 14th days but attempts to recover virus from them failed; the fourth animal survived but its serum was not tested for antibody. Four other *Claviglis murinus* were inoculated subcutaneously with 13,200 LD₅₀ each of the same virus. Three of these became sick and were sacrificed for passage on the 5th, 6th and 7th days but virus was not recovered from either brain or liver. The fourth animal remained well. A protection test, employing a 10 per cent suspension of its liver in lieu of serum, demonstrated no neutralizing antibody. These results indicate that *Claviglis murinus* is not highly susceptible to pantropic yellow fever virus.

Eight multimammate rats (*Mastomys coucha*) were inoculated intracerebrally with 3,970 LD₅₀ of the pantropic virus isolated from *Aedes simpsoni*. One of them was sacrificed for passage when it was moribund on the 13th day, but virus was not recovered from its brain. The other 7 remained well. It was necessary

to pool their "convalescent" sera for protection tests: 2 pools, 1 of sera from 3 animals and the other from 4, both gave protective results. Inasmuch as there were no preinoculation tests on these animals, the final protective sera can not be said to have resulted from the inoculations.

Seven other *Mastomys coucha* were inoculated subcutaneously, each with 13,200 LD₅₀ of the same virus. All of them remained well. No "convalescent" serum was obtained for protection test from 2 of these, but the others yielded the following results.

- 1 single specimen, non-protective.
- 1 pool of 2 sera, inconclusive.
- 1 pool of 2 sera, protective.

The results seem to indicate a low degree of susceptibility for these animals and the probability that the virus does not propagate well in them.

Nine thicket rats (*Thamnomys dryas*) were inoculated intracerebrally with 3,970 LD₅₀ of the same pantropic yellow fever virus. Two died of unknown causes on the 3rd and 4th days. The other 7 survived without becoming ill, and 10 per cent saline extracts of their livers all gave protective reactions in the yellow fever protection test.

Nine *Thamnomys dryas* were inoculated subcutaneously with 13,200 LD₅₀ of the same virus. All of them remained well. Extracts of their livers were used in lieu of serum in the yellow fever protection test; 2 were protective, 3 gave inconclusive results and 4 were non-protective. Since no preinoculation protection tests were made on the animals of this species, it is impossible to state that all those observed to be immune at the termination of the experiment acquired their immunity as a result of the inoculations. However, if one assumes that they did, it would appear that yellow fever virus is more effective in this species (and possibly in *Mastomys coucha*) when introduced intracerebrally than when given subcutaneously. The results do not indicate a high degree of susceptibility for *Thamnomys dryas*.

Experiment 3. A non-immune adult hyrax (*Procavia* sp. indet.), one of 4 captured in the environs of El Obeid, Anglo-Egyptian Sudan, was inoculated subcutaneously with 1.0 ml. of 1 in 10 dilution of rehydrated yellow fever virus, Asibi strain. The titre of the virus was such that this dose represented 556,110 mouse-intracerebral LD₅₀. The animal was bled daily by cardiac puncture and tests for circulating virus were made. Except on the 6th day, when virus was present in small quantity (120 LD₅₀ per ml. of serum), the tests for circulating virus were negative. The animal showed no obvious signs of illness at any time following the inoculation, although it had temperatures 2°F. or more above its normal average on the 4th to 8th days after inoculation. Serum taken on the 16th day gave an immune reaction in the yellow fever protection test.

Of the other 3 hyraxes received from Dr. Horgan, 1 was immune on arrival at Entebbe. The remaining 2 were non-immune but they died before they could be employed in susceptibility tests. The observation that one animal was immune when captured indicates that this species may acquire infection in nature, but the results of the inoculation experiment suggest that hyraxes of this variety

are not of great epidemiological importance, as the animal circulated so small an amount of virus that there is little probability that it could have transmitted enduring infection to a blood-sucking arthropod.

Experiment 4. A juvenile non-immune Uganda red hog (*Potamochoerus porcus*) was inoculated subcutaneously with 1.0 ml. of 1 in 1,000,000 dilution of rehydrated yellow fever virus, strain isolated from *Aedes simpsoni* in 1942. This dose represented 92 mouse-intracerebral LD₅₀. The animal showed no obvious clinical reaction and exhibited no significant elevation of temperature. Tests were made daily for circulating virus, the 5th day test showing a trace (2 of 6 mice receiving undiluted serum succumbed) and the other days' tests none. Serum taken on the 10th day contained neutralizing antibody, showing the development of immunity in consequence of the inoculation. The results indicate that this species of bush pig probably is unimportant in the epidemiology of jungle yellow fever.

Experiment 5. A juvenile female non-immune leopard (*Felis pardus*) was inoculated subcutaneously with 1.0 ml. of 1 in 100 dilution of rehydrated yellow fever virus of the strain isolated in 1942 from *Aedes simpsoni*. This dose represented 132,200 mouse-intracerebral LD₅₀ of virus. The animal exhibited no objective signs and had no febrile reaction as result of the inoculation. Among all the mice used in the tests for circulating virus, only 1 of a group of 6 which received undiluted 7th day serum of the leopard succumbed and it is not certain that this death was caused by yellow fever virus. Serum taken on the 6th day gave an inconclusive reaction in the yellow fever protection test, while 7th-, 8th- and 9th-day specimens were each protective. The failure of this animal to circulate virus in quantity sufficient to infect vector insects suggests that *Felis pardus* probably does not participate in natural cycles of infection with yellow fever virus.

DISCUSSION

The small size of some of the rodents included in this study imposed technical obstacles which made exhaustive experiments impossible. The difficulty in obtaining other species (hyrax, bush pig and leopard) prevented adequately comprehensive experiments on these. Nevertheless, sufficient tests for circulating virus were made on 2 species of rodents (*Arvicanthus abyssinicus* and *Lemniscomys striatus*) to warrant the doubt that these 2 species ever participate in natural cycles of infection with yellow fever virus. The single specimen of another species of rodent (*Cricetomys gambianus*) on which a satisfactory result was obtained, and the hyrax, bush pig and leopard gave results which are not characteristic of susceptible or epidemiologically important species. The tests on the other 4 species of rodents, while not wholly conclusive, also seem to indicate low susceptibility to pantropic yellow fever virus.

SUMMARY

Tests of susceptibility to pantropic yellow fever virus were made on 7 species of African rodents, a hyrax, a Uganda red hog and a leopard. Although some of the results have to be regarded as inconclusive, none of the species was found

to be highly susceptible and it seems likely that none of them is of much importance in the epidemiology of jungle yellow fever.

It was found that a sterile suspension of liver tissue, clarified by centrifugation, suffices in lieu of serum for use in the yellow fever protection test when an adequate quantity of serum is not obtainable.

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III. POTTOS AND GALAGOS¹⁶

K. C. SMITHBURN

When it became apparent that an arboreal vector was responsible for the transmission of yellow fever in extra-human cycles of the disease (1) attention had to be given to arboreal animals as possible vertebrate hosts of the virus. Thus our interest in pottos and galagos (or bush babies) was aroused. Both are highly arboreal, and the suborder *Lemuroidea*, to which both belong, includes many species which are distributed over large areas in central Africa and the adjacent islands on both the east and west coasts of the continent. Some occur in areas of true primaeval forest, while others are found in bush or open savannah. Therefore, if they were generally susceptible to yellow fever they could be of epidemiological importance in regions where monkeys are scarce or do not occur.

MATERIALS AND METHODS

The general methods employed in the study were described at length in the first section and will not be given here. Only such procedures as were different from those employed in the studies on monkeys will be described.

Animals. The pottos were captured either in Bwamba County in western Uganda, or in the Mengo District. They were supplied to us by Dr. A. J. Haddow, of our own staff, and Capt. C. R. S. Pitman, Game Warden of the Protectorate Government, whose interest and collaboration are gratefully acknowledged.

The galagos all came from the Gede or Kilifi Forests of the Kenya coastal area and were supplied to us by Dr. James M. Liston, formerly Port Health Officer at Mombasa, and Dr. P. C. C. Garnham, formerly Specialist, Senior Parasitologist, Kenya Medical Services, to both of whom we are much indebted.

Two species, *Perodicticus potto ibeanus* Thomas and *Galago crassicaudatus lasiotis* Peters, were studied. The results indicate that both may be of considerable epidemiological importance.

Mosquito Transmission. The one insect transmission experiment included in these studies was done to determine whether the known laboratory vector,

¹⁶ by K. C. Smithburn.

Aedes (Stegomyia) africanus Theobald, the mosquito then most strongly suspected (1) and now known (2) to be a forest vector of yellow fever, is capable of transmitting the virus from one galago to another. Wild-caught female mosquitoes were placed in gauze-covered glass tubes 1 inch in diameter and were allowed to take blood from an infected galago. The serum of the latter was titrated for virus immediately after the mosquitoes had fed. During the extrinsic incubation period, the insects were kept in the gauze-covered tubes in a battery jar over saturated NaCl solution within a controlled temperature cabinet at 28°C. The infecting and transmitting feedings were given in the same manner.

Tests for virus in mosquitoes were made immediately after the infective feeding and following the transmitting feeding. Individual insects were killed with tobacco smoke, ground in a mortar and suspended in 1.0 ml. each of 10 per cent non-immune serum in physiological saline. The suspensions were spun for 20 minutes in the angle centrifuge at about 2500 r.p.m., and groups of 6 mice were inoculated intracerebrally with 0.03 ml. portions of the supernate.

Serial Transmission by Inoculation. The final experiment with galagos involved the animal-to-animal transmission of the virus by serial subcutaneous inoculations of serum, starting from an animal inoculated with potent pantropic virus. At the time the subinoculations were done they constituted "blind" passages, as the amount of virus present in each specimen of serum was not known. Intracerebral titrations were done on specimens of serum used for subinoculation, employing groups of 6 mice for each dilution of serum. End points were calculated by the method of Reed and Muench (3) and the end point dilution was regarded as containing 1 LD₅₀ per unit of inoculum (0.03 ml.). In this way the dose of virus subinoculated was determined in each instance.

Immunity Surveys in Galagos. As a part of our general epidemiological studies on yellow fever in East Africa, we have conducted surveys of immunity in various species of wild animals. The latter have been obtained by purchase, by trapping and by hunting. The protection tests have been done by methods now in standard use in this laboratory (4).

Through the courtesy and collaboration of friends in Kenya considerable numbers of galagos were caught in the Gede and Kilifi Forests, and the animals themselves or samples of their blood were sent to us. All the animals which survived long enough for blood specimens to be obtained were tested for immunity to yellow fever. Some of the non-immunes were then used in the susceptibility tests, the details of which follow.

Very few specimens of pottos have come to hand, and none of the 5 tested (all captured in Uganda) has been found to be immune to yellow fever.

PROCEDURES AND RESULTS: POTTOS

Susceptibility Tests. As a preliminary experiment the first potto tested was given, subcutaneously, 132,000 mouse-intracerebral LD₅₀ of the strain isolated in 1942 from *Aedes simpsoni* (5). Subsequently 2 others were inoculated subcutaneously with 120 mouse-intracerebral LD₅₀ of another strain of pantropic

yellow fever virus isolated in 1944 from *Aedes* mosquitoes (5). Temperatures of the animals were taken twice daily (once on Sundays and holidays), and each was tested daily for circulating virus.

None of the pottos exhibited a febrile reaction as a result of its inoculation; in fact, the observed temperatures were frequently in the subnormal range. None died of yellow fever, and each survived during the 10-day period of the tests for circulating virus. The 2 which received the smaller doses of virus suffered intercurrent infections and succumbed on the 12th and 13th days after inoculation. By this time, however, the serum of 1 had been negative for virus for 5 days and the animal had had demonstrable protective antibody in its serum for 3 days. Yellow fever may have been contributory to, but was certainly not the sole cause of, death.

Each of the pottos exhibited virus in its circulation for at least 4 days and in 1 the tests were positive from the 3rd to 10th days inclusive, as shown in Table 10. The maximum titres were not very high but they were probably high enough in each animal to infect mosquitoes on one or more days.

Two of the 3 pottos showed protective antibody in their sera resulting from the inoculation. The serum of the third was still devoid of antibody on the 10th day; no tests were made thereafter as the animal died before the result of the 10th-day test was known.

GALAGOS

Immunity Surveys. Sera of 66 galagos captured in the Gede and Kilifi Forests in the Kenya coastal area were examined by the yellow fever protection test, with results as follow:

Non-immune.....	53
Inconclusive.....	4
Protective (immune).....	9

Thus, of the 62 animals giving decisive results, 9 (14.5 per cent) were immune. This observation was of interest not only in revealing that galagos acquire infection with yellow fever virus under natural conditions, but also in showing that yellow fever occurs among wild primates in the Kenya coastal area. The latter fact was confirmed in 2 ways: (a) by finding that immunity to yellow fever occurs in other species of primates (*Cercopithecus aethiops johnstoni* Pocock) captured in the same forests, and (b) by the observation that galagos, whose sera gave positive results in protection tests were completely resistant to yellow fever virus, while those whose sera gave negative reactions were susceptible.

Although the foregoing results showed that galagos acquire immunity to yellow fever in nature, it could not be assumed without experimental evidence that they are capable of passing on yellow fever virus to vector insects, thus serving as participants in natural cycles of infection. The experiments which follow were undertaken to elucidate this point.

Susceptibility Tests. Twenty-six galagos were inoculated with pantropic yellow fever virus or infected by the bites of mosquitoes, as shown in Table 1.

TABLE 10

Summary of results in tests of susceptibility of *pollos* and *galagos* to yellow fever virus

ANIMAL NO.	SPECIES	INFECTED WITH		FEBRILE REACTION		CIRCULATING VIRUS			SERUM FIRST PROTECTIVE	REMARKS
		Virus strain	Dose, mouse LD ₅₀	Elevated temp., days	Maxi- mum °F.	Day of maxi- mum	Days tested	Days present		
2	<i>Perodicticus potto ibeanus</i>	Uganda, <i>A. simpsoni</i> , '42	132,000	No	—	—	1 to 10	3 to 0, 8*	day	
741	<i>Perodicticus potto ibeanus</i>	Uganda <i>Aléca</i> , '44	120	No	—	—	1 to 10	3 to 10	9	Died 12th day†
862	<i>Perodicticus potto ibeanus</i>	Uganda <i>Aléca</i> , '44	120	No	—	—	1 to 10†	4 to 7	Negative 10th	Died 13th day†
482	<i>Galago crassicaudatus lasiotis</i>	Asibi	556, 110	3	102.8	3	1 to 7	1 to 5	10§	
483	<i>Galago crassicaudatus lasiotis</i>	Asibi	556, 110	1 to 3	103.6	1	1 to 7	1 to 4	10§	Sacrificed 5th day
507	<i>Galago crassicaudatus lasiotis</i>	Asibi	556	4	102.2	4	5	5	3,330	Died 4th day
508	<i>Galago crassicaudatus lasiotis</i>	Asibi	556	No	—	—	3	3	11,500	Died 5th day
570	<i>Galago crassicaudatus lasiotis</i>	Asibi	460	4	102.4	4	1 to 5	2 to 5	70,590,000	
571	<i>Galago crassicaudatus lasiotis</i>	Asibi	460	5 to 10	103.0	5	1 to 10	1 to 7	10,550,000	
574	<i>Galago crassicaudatus lasiotis</i>	Asibi	3,396	3	103.0	3	1 to 5	1 to 5	83,916,000	
575	<i>Galago crassicaudatus lasiotis</i>	Asibi	3,396	3, 4	104.2	4	5, 7	5, 7	105,501,000	
580	<i>Galago crassicaudatus lasiotis</i>	Asibi	3,396	3	103.8	3	4	4	58,275,000	
583	<i>Galago crassicaudatus lasiotis</i>	Asibi	3,396	3	103.2	3	4, 10	4	126,540	
584	<i>Galago crassicaudatus lasiotis</i>	Asibi	3,396	No	—	—	4, 5	4, 5	5,927,400	
588	<i>Galago crassicaudatus lasiotis</i>	Asibi	3,396	2 to 4	105.2	4	4	4	47,280,000	
573	<i>Galago crassicaudatus lasiotis</i>	Asibi (ox 588)	2 <i>A. affri-</i> <i>canus</i>	3	102.8	3	3 to 5	3 to 5	13,153,500	Transmission by 2
603	<i>Galago crassicaudatus lasiotis</i>	Asibi	2,051	3	104.0	3	1 to 10	2 to 0	Not titrated	<i>A. africanus</i>
604	<i>Galago crassicaudatus lasiotis</i>	Asibi	2,051	3	101.2	3	1 to 5	3 to 5	Not titrated	Died 11th day
624	<i>Galago crassicaudatus lasiotis</i>	Asibi 1st passage	2,051	2, 4, 5	102.2	4	4 to 10	4, 5	10§	Died 5th day†
630	<i>Galago crassicaudatus lasiotis</i>	Asibi	2,051	5, 6	101.8	5	1 to 9	2 to 0	21,000	
640	<i>Galago crassicaudatus lasiotis</i>	Asibi 2nd passage	2,100	4	101.2	4	1 to 10	3 to 5	10,556,100	
641	<i>Galago crassicaudatus lasiotis</i>	Asibi (ex 624)	2,100	3	101.4	3	4 to 10	4 to 7	330,000	
625	<i>Galago crassicaudatus lasiotis</i>	Asibi 3rd passage	131,535	2	103.0	2	1 to 4	1 to 4	1,315,350	Died 14th day
638	<i>Galago crassicaudatus lasiotis</i>	Asibi (ex 641)	131,535	3 to 0	103.6	4	4 to 10	4	1,055,010	Died 4th day†
636	<i>Galago crassicaudatus lasiotis</i>	Asibi 4th passage	0.033	7, 8	102.0	7	3 to 9	0 to 0	5§	Died 9th day
642	<i>Galago crassicaudatus lasiotis</i>	Asibi (ex 638)	0.033	3 to 5	103.4	5	4, 5	4, 5	Negative 9th	
616	<i>Galago crassicaudatus lasiotis</i>	Asibi	14,552	2 to 4	103.0	3	1 to 5	1 to 5	105,501,000	Died 6th day†
637	<i>Galago crassicaudatus lasiotis</i>	Asibi 5th passage	14,552	3, 4, 7 to 11	103.0	10	1 to 10	1 to 5	8,301,000	Died 6th day†
643	<i>Galago crassicaudatus lasiotis</i>	Asibi (ex 642)	14,552	3 to 5	104.6	4	3 to 7	3 to 0	333,000	
									7,569,000	Died 8th day

* Seventh day serum caused no deaths in mice, but virus was again present in small quantity on the 8th day.

† Illness complicated by extraneous factors.

‡ Blood not obtained on 3rd and 8th days.

+ Indicates that end point was not reached in the highest dilution tested.

§ No prior postinoculation tests.

|| No test previous day.

¶ Peak titre obviously occurred prior to 4th day, during which time no tests were made.

Experiment 1. Galagos M 482 and 483 received large doses and their sera were tested for circulating virus each day for a week. Insufficient dilutions of the sera were tested on the days of their peak titres and the maxima were not determined, but the observed values were relatively high. Both animals exhibited mild febrile reactions, but neither became seriously ill and both survived the experiment. Each was bled for protection test on the 16th day and both their sera were protective.

An interesting incidental development was observed in this experiment which later proved to be a common phenomenon in infected galagos. From the 4th day onward the sera of both galagos were moderately opaque and bright emerald green in color, deepest on the 4th and 5th days and paler green on the 6th and 7th. Concomitantly there was prolongation of the clotting time. The source of the green pigment was never determined, but the phenomenon was repeatedly observed in subsequent tests on other animals.

Experiment 2. The foregoing preliminary experiment having indicated that galagos might be highly susceptible, 2 others, M 570 and M 571, were inoculated with 460 LD₅₀ each of Asibi virus in order to carry out a more critical test.

Galago M 570 had a rise in temperature on the 4th day and appeared ill. It was weaker the next morning and died during the 5th day. Galago M 571 first had fever on the 5th day, at which time it appeared listless and did not eat well. By the 7th day it began to show improvement, although its temperature was still elevated (Table 2). It survived the infection, and protection tests revealed that demonstrable antibody was present on the 7th day. The temperature records and the results of tests for circulating virus in the 2 animals are shown in Table 11.

The results of this experiment showed that yellow fever virus multiplies in the tissues of galagos at least as well as in those of the most susceptible African monkeys. They also left little doubt that an infected galago could infect vector insects. Nevertheless, it was decided to determine their ability to do so by actual test.

Experiment 3. Six non-immune galagos, Nos. M 574, 575, 580, 583, 584 and 588, were each inoculated subcutaneously with 3,396 LD₅₀ of Asibi virus. Tests for circulating virus were made daily on galago M 574 until it died; the others were tested only on the days when the peak titres of circulating virus were expected to occur. On the 4th day *Aedes (Stegomyia) africanus* Theo. mosquitoes were allowed to bite galago M 588; 3 of the fed insects were tested individually for virus immediately after the feeding, with positive results in each instance; the remaining infected mosquitoes were stored in the controlled temperature cabinet at 28°C.

Only 1 of these 6 galagos survived. The others died on the 5th to 7th days and each exhibited gross and/or microscopic lesions of yellow fever (see section on Lesions in Galagos). The tests for circulating virus showed that the Asibi virus had propagated vigorously in each animal; the observed titres exceeded 1,000,000 LD₅₀ per ml. of serum in each of the 5 animals which succumbed to the infection. Even the animal which survived, M 583, had virus in its serum on

the 4th day in such amount that, had *Aedes aegypti* been allowed to feed on it, each mosquito would have acquired approximately 316 mouse-intracerebral LD₅₀ of virus.

Only 5 of the *A. africanus* remained alive 14 days after being infected on galago M 588. They were offered non-immune galago M 573. Only 2 fed; 1 of these and 2 others which refused galago 573 were dead the following day and they were tested separately for virus, with positive results in each instance.

Three days after being bitten by the 2 infected *A. africanus*, galago M 573 had a rise in temperature and appeared moderately ill. After 2 days of obvious illness the animal began to improve and it survived the experiment. Tests for circulating virus were made on the 3rd to 5th days with results as follow:

Day of test	LD ₅₀ virus per ml. serum
3	13,153,500
4	5,827,400
5	47,286

TABLE 11

The reactions of galagos M 570 and M 571 to inoculation with small doses of Asibi virus

DAYS AFTER INOCULATION	GALAGO M. 570		GALAGO M. 571		
	Maximum tem- perature	LD ₅₀ virus per ml. serum	Maximum tem- perature	LD ₅₀ virus per ml. serum	Protection ratio* of serum
	°F.		°F.		
1	100.6	0	100.4	0	—
2	100.2	4,396	100.2	<33	—
3	100.6	10,556,100	100.0	3,300	—
4	102.4	76,590,000	100.8	10,556,100	—
5	101.0†	47,286,000	103.6	4,728	0/7
6			101.0	7,659	3/8
7			102.2	33	7/8
8			101.6	0	8/8
9			101.6	0	—
10			102.0	0	8/8

* Numerator indicates the number of mice surviving, the denominator the number inoculated.

† Animal died this day.

A protection test done with 12th-day serum of galago 573 showed that the animal was then immune.

This experiment confirmed the earlier observation that yellow fever virus propagates vigorously in galagos and appears in their sera in high concentration. It also showed that the virus can be readily transmitted to them by the bites of *Aedes africanus* and that the mortality in galagos experimentally inoculated with pantropic yellow fever virus may be high.

Experiment 4. Four galagos were inoculated subcutaneously, each with 2,051 LD₅₀ of Asibi virus. The serum of M 639 was titrated for virus content each day; undiluted sera of M 603 and M 604 were tested daily, but no titrations

were done; M 624 was reserved for 4th-day passage to another pair of galagos, M 640 and M 641, and its serum was titrated daily from the 4th to the 10th day.

Galagos M 640 and M 641 received 1.0 ml. of 1 in 10 dilution of 4th-day serum of galago M 624, representing 2,100 mouse-intracerebral LD_{50} of virus. The serum of galago M 640 was titrated for virus daily from the 1st day onward, but titrations were not started on M 641 until the 4th day, when its serum was used for subinoculation.

Galagos M 625 and M 638 received subcutaneous inoculations of 1.0 ml. each of a 1 in 10 dilution of the 4th-day serum of galago M 641, which represented 131,535 LD_{50} of virus. The serum of M 625 was titrated daily, but it died of hemopericardium on the 4th day. The 4th-day serum of M 638 was used for subinoculation and titrations were made daily from that day onward. Galago M 625 had its peak titre of circulating virus on the 2nd day, and the agent had all but disappeared from its serum by the 4th day (Table 12). No titrations were done on the serum of galago M 638 during the first 3 days but the probability is that it behaved similarly. Although its 4th-day serum was very low in virus content, passage to galagos M 636 and M 642 was successful.

Galagos M 636 and M 642 each received 1.0 ml. of 1 in 1,000 dilution of the 4th-day serum of galago M 638. The undiluted serum of M 638 caused the death of only 3 of 6 inoculated mice, and the dilutions of this serum caused no deaths in mice. The inoculum for M 636 and M 642 therefore represented only 0.033 mouse-intracerebral LD_{50} of virus. The serum of M 636 was tested (but not titrated) for virus daily until the animal died on the 9th day. The serum of M 642 was titrated on the 4th day, when it was used for subinoculation, and again on the 5th day; the animal was found dead on the morning of the 6th day.

The 5th and final serial passage in galagos was made to M 615, M 637 and M 643, each of which received subcutaneously 1.0 ml. of 1 in 1,000 dilution of the 4th-day serum of M 642. This represented 14,552 mouse-intracerebral LD_{50} of virus. Titrations of sera for virus content were done on these 3 animals as shown in Table 3. Galagos M 615 and M 643 died of yellow fever; M 637 survived.

The temperature reactions, results of tests for circulating virus and the protection test data on the animals in this passage series are summarized in Table 3. Those which exhibited the "green serum phenomenon" are indicated and the time of deaths is recorded.

The high values for circulating virus and the high mortality in the passage animals are noteworthy, but the most interesting observation in this experiment is the fact that galagos M 636 and M 642 became infected following the inoculation of a dose of virus considerably smaller than that required to infect mice. This is important not only in showing that mice do not respond to a single unit of virus, but also in pointing to the much higher susceptibility of the galago.

MORTALITY IN GALAGOS WITH EXPERIMENTAL YELLOW FEVER

One of the galagos, M 507, was sacrificed for histological studies, but all others were allowed to survive or die as they would. Fifteen of the remaining 25 succumbed; 3 of the deaths were unquestionably caused in part by technical

TABLE 12
Results obtained by serial passage of *Asibi virus in galagos*

	603	604	624	639	640	641	625	638	636	642	615	637	643
	1				2		3			5			
Galago no.													
Passage.										4			
Source	Inoculum												
LD ₅₀	Desicated Virus				M 624		M 641		M 638		M 642		
	2651				2100		131,535		0.033		14,552		
Tests for circulating virus, day	0/3*	0/5		0	0		1,050 ⁺				800 ⁺	707 ⁺	
1	4/6	0/6		59	33,300		1,055,010 ⁺				333,000	333,000	
2	0/6	0/6		1,918,080	333,000		24,042				8,301,000	114,885	96,570
3	0/6	0/6		10,550,100	1,005	1,315,350	<33	33	0	0	14,552,100	2,404	7,059,000
4	0/6	0/6	21,000†	5,827	0	3,330	0	0	0	0	105,501,000	<33	6/6
5	3/6	1/5	833	0	0	330	0	0	0/6	0	100	0	2/5
6	1/6	0	33	0	0	330	0	0	0/6	0	0	0	0/6
7	0/6	0	0	0	0	47	0	0	0/6	0	0	0	0/6
8	0/6	0	0	0	0	0	0	0	0/6	0	0	0	0/6
9	0/6	0	0	0	0	0	0	0	0/6	0	0	0	0/6
10	0/6	0	0	—	0	0	0	0	0/6	0	0	0	0
Died or survived	D	D	S	S	S	D	D	S	D	D	D	S	D
Day of death	11	5			14	4			9	6	5	8	8
Serum protection ratio‡ (day)				1/8	1/7	3/8		8/8	1/8			7/8	8/8
5				8/8	4/6	4/6		6/8	0/8			7/7	7/7
6				8/8	4/4	4/7		6/7	0/6			7/8	8/8
7				0/7	4/4	8/8		0/7	0/7			8/8	
8	7/8		8/8				0/7						
9						+			+		+	+	+
10													
Green serum		+											
Fever, days	3	3	2, 4, 5	5, 6	4	3	2	3 to 6	7, 8	3 to 5	2 to 4	3, 4, 7 to 11	3 to 5

* Mortality ratio, the numerator indicating the number of mice which died, the denominator the number inoculated.

† Figures show virus content of serum in mouse-intracerebral LD₅₀ per ml.

+ Indicates end point not reached owing to insufficient dilutions being tested.

§ Numerator shows number of mice surviving, the denominator the number inoculated.

procedures, as galagos M 604 and M 625 exhibited hemopericardium and M 642 showed hemothorax resulting from cardiac punctures. Furthermore, galago M 615 exhibited abscesses in its liver which doubtless were a contributory cause of death but were not caused by the virus. If these animals be excluded from consideration, the mortality rate was 11 out of 21, or 52.4 per cent.

LESIONS INDUCED BY YELLOW FEVER VIRUS IN GALAGOS

The lesions observed at autopsy in galagos were similar in every way to those which are characteristic of the disease in man or in rhesus monkeys. Of the 16 which came to autopsy (1 of which was sacrificed and 3 of which died from hemorrhage), 7 exhibited livers to which the term "boxwood" could be accurately applied. One of these was the sacrificed animal and another was one which died of hemothorax. Livers of the remaining animals were variously describable as yellow-brown or red-brown in color, with one exception. The latter, M 641, died on the 14th day, and its liver was nearly normal in color; however, the microscopic studies on this animal revealed the presence of specific changes. None of the animals showed enlargement or noteworthy shrinkage of the liver. In most instances the tissue appeared moderately greasy on section. Hemorrhages into the gastric mucosa and/or black altered blood in the stomach contents were noted in 7 of the 16 animals.

Microscopically the hepatic lesions were quite different from those occurring in man or in rhesus monkeys. None of the characteristic acidophilic necrotic cells (Councilman bodies) were found, and the inclusions frequently seen in rhesus monkeys (and less commonly in human liver tissue) did not occur. The livers of the galagos, with one exception, M 641, showed fatty degeneration, usually widely distributed and extensive. There was necrosis of hepatic cells with a definitely midzonal preference, but the affected cells were not hyperacidophilic. Instead, they took both the hematoxylin and the eosin poorly and gave a pale, washed-out appearance. No noteworthy nuclear changes were seen. Infiltration of the tissue with mononuclear cells was a constant and pronounced feature. Hyperemia and even hemorrhages were commonly present. The evolution of the hepatic lesions in galagos is evidently similar to that which occurs in humans, as galago M 641, which died on the 14th day, exhibited lesions similar to those found by Villela (6) in human beings with yellow fever in whom death was delayed. The liver of galago M 641 showed no fatty degeneration; but there was moderate disorganization of the trabeculae, hyperplasia of Kupffer cells, and infiltration with mononuclears, and numerous "ochre-colored granular bodies" were present. The latter were concentrated in the central and mid-zones of the lobules where trabecular disorganization was most marked. They appeared to be large phagocytic cells vigorously engaged in dealing with the debris of necrotic tissue.

SUMMARY

Tests of susceptibility to yellow fever virus were made in *Perodicticus potto ibeanus* and *Galago crassicaudatus lasiotis*. The potto was found to be susceptible, in that the virus propagates in it and is present in its blood in con-

siderable quantity for several days. The galagos were even more highly susceptible, exhibited circulating virus in higher titre and about half of them died of the disease. It was found that they may be infected by a dose of virus which is much too small to cause specific infection in white mice. Yellow fever virus was transmitted from one galago to another by the bites of 2 *Aedes africanus*. Naturally-acquired immunity to yellow fever was found in galagos captured in the coastal area of Kenya Colony. The results indicate that some species of this genus may be of considerable importance in the epidemiology of extra-human cycles of the disease.

About 50 per cent of infected galagos exhibit an emerald green coloration of the serum for 2 to 5 days, coinciding roughly with the period of circulating virus.

The lesions induced by yellow fever virus in galagos, although similar in the gross to those in man and rhesus monkeys, differ in their microscopic aspects. Neither inclusion bodies nor acidophilic necrosis of hepatic cells were observed in them; the microscopic lesions are therefore less conspicuous in galagos.

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PHTHALYLSULFACETIMIDE (THALAMYD) IN CHOLERA

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Since cholera is characterized by acute salt and water depletion, and severe general toxemia, the therapeutic problems are to restore fluid and salt, and to eradicate the infecting organism. In addition, special measures are being employed to protect body systems against the toxemia, such as the use of testosterone for its effect on the kidney (8).

In 1947 cholera appeared in Egypt for the first time since 1902, reaching epidemic proportions during September and forming a total of 21,661 cases by the end of December (14). The mortality was 10,696 or 49.4 per cent. During this epidemic, we were able to treat more than one hundred patients with phthalylsulfacetimide, but, owing to well known difficulties prevailing in any cholera epidemic, we have complete records for only forty. In this series of forty cases, there was one death, or 2.5 per cent. For reasons which will be made plain, we do not stress comparative mortality figures, nor insist upon the validity of statistics purporting to evaluate a drug during a raging epidemic.

Clinically, cholera may vary from a mild diarrhea to fulminating catastrophe with collapse and death, accounting for the varying mortalities (from 5 per cent to 80 per cent) cited in different epidemics; moreover, lower figures prevail as a rule toward the end of an epidemic. The largest single factor in the reduction of mortality, as all are agreed, is the intensive restoration of salt and water balance. More recently, sulfonamides have been used against the comma vibrio. The drug most commonly given has been sulfaguanidine (4, 6, 7, 9-11), but succinylsulfathiazole (11), sulfadiazine (3, 4, 11), and other preparations (1) have also been used. Following the work of Chopra *et al.* (2) in 1941, various compounds have been pronounced effective (1, 2, 12, 13, 16, 17). The vibrio is not sensitive to penicillin, and streptomycin has been found ineffective (15).

It is obvious from the aggregate of reports that the sulfonamides which have been used thus far offer no significant advantage over the established use of fluid and salt; and, moreover, that wide variability characterizes different epidemics in different regions and years. Thus, only 25 per cent of untreated patients survived in one series (10), as against figures of 50 per cent to 60 per cent in others (11); whereas in hospitalized patients receiving adequate and competent supportive therapy, from 60 per cent to almost 100 per cent (4) might be expected to survive.

The present series is unaccompanied by presumably equivalent control cases, the significance of which would be, it is admitted, only problematical. The overall mortality in the present epidemic, 49.4 per cent, is cited as a first approximation, subject to hypothetical analysis on the basis of the proportion of cases treated and untreated, and other factors, all of which remain unknown.

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Interest in the possible use of phthalylsulfacetimide in cholera arose from the demonstration of its exceedingly high activity against the cholera vibrio *in vitro* (19).

In addition to its bactericidal action, phthalylsulfacetimide has the remarkable property of being absorbed by diffusion into the several layers of the intestinal wall (18), yet of being "unabsorbable" in the sense that blood concentrations of the drug cannot be detected in man following therapeutic dosage.

These findings readily suggested the applicability of phthalylsulfacetimide to the treatment of cholera, since it could be presented in high concentration at the very locus of infection, neither reaching it incidentally through a high systemic concentration, with the toxicity this implies, nor merely washing it superficially by admixture with the contents of the bowel.

The treatment of patients with this compound confirms its vibriocidal activity by demonstrating a consistent, early disappearance of the vibrio from the stools.

Phthalylsulfacetimide exhibits the peculiarly low toxicity of the sulfacetimide series of compounds, as compared with the recognized toxicity of N¹-substituted sulfanilamides (such as sulfadiazine, sulfaguanidine and sulfathiazole).

The forty cases comprised thirty-one males and nine females; all except four were adults. Depending upon clinical criteria, and especially the physical findings, they were graded as mild (8 cases), moderate (17 cases) or severe (15 cases). A severe case was one that showed signs of dehydration, shock, and collapse, with which was associated a loss of elasticity of the skin resulting in "washer-woman" palms; the eyes were sunken, the voice was hoarse and feeble, and the respirations rapid and shallow. The pulse was rapid and weak, the heart sounds distant, and the blood pressure very low. Cyanosis of the skin and mucous membranes was common. Temperatures were usually below normal. There were oliguria with albuminuria and, in very severe cases, anuria. The blood specific gravity ranged between 1046 and 1074, with average 1060. The presumptive diagnosis of cholera was established by the circumstances of the epidemic and the clinical condition of the patient. The presence of *V. comma* in the stools was accepted as firm diagnostic evidence of the disease, although half the patients, with obvious clinical cholera, failed to show typical, agglutinable, non-hemolytic *V. comma*. It may be of interest that the greatest number of patients had not been inoculated against cholera at all (18 cases) or had received only a single dose of vaccine (14 cases), while the remainder had received a second dose from one to four weeks before the onset.

In every case, save one, phthalylsulfacetimide was given in a dosage of 5 Gm. initially and 1 Gm. every two hours day and night for five days, or a total of 64 Gm., beginning on the first or second hospital day in most patients. In all cases a high level of fluid administration was maintained. The patients received 1 to 5 liters of saline, or saline and sodium bicarbonate solution, daily, usually by vein as well as fluids orally. In no case was there any evidence of toxic action attributable to phthalylsulfacetimide: a question of surpassing gravity in patients such as these, who are virtually moribund because of the basic disease and can scarcely tolerate further insult due to therapy with drugs

of even moderate systemic toxicity. This established beyond question the safety of the new drug.

Cholera vibrios disappeared from the stools promptly in most of the patients. Clinically there was a gradual improvement characterized by the cessation of vomiting and diarrhea, closely followed by the passage of formed stools. The general condition improved until recovery ensued and the patients were discharged to convalescent care, requiring an average of fourteen hospital days in the present series.

The patient who died was a man of 35 who was admitted after a single day of illness and was anuric on entry, with severe symptoms and blood specific gravity 1065. On admission the blood urea was 147 mg. per 100 cc. Vibrios were not found in the stools at any time. Following the full five day course of phthalylsulfacetimide he passed semi-solid and then formed stools, vomiting had ceased. However, on the tenth day the blood urea rose to 170 mg. per 100 cc., and he died on the eleventh day. Autopsy showed pulmonary edema and renal enlargement.

The high activity of the drug *in vitro* plus the fact that it promptly rid the stools of the invading organism in clinical patients, encouraged us to attempt prophylactic use of the drug on a section of the population in the endemic area. While theoretically this seems a good plan, in practice it simply did not work, primarily because continued observation of the selected populace was impossible, so that we had no definite knowledge that the drug was being taken according to directions. However, we were able to isolate and keep under constant observation a small group. Stools of three thousand contacts were examined. Some of these showing the presence of the vibrio were treated with phthalylsulfacetimide 0.2 grams per kilo body weight daily. The organism promptly disappeared from the stool within twenty-four to forty-eight hours. This is of limited significance since the cholera vibrio also disappeared from the stools of some of the untreated control group often within about the same period of time. This does not, however, vitiate the importance of eradicating the organism by a positive prophylactic effort, since at least one of these untreated patients went on to develop fulminating cholera, whereas none of the treated patients did so during the period of observation.

Mass prophylaxis of a populace could be successfully carried out provided adequate, organized personnel were available from trained groups, such as the military. It is postulated that if every member of the population in an endemic area were given the drug in a dosage of 0.2 grams per kilo of body weight daily for thirty days, the epidemic would be terminated. Such a procedure should have considerable military significance since an outbreak of cholera in any military group could be promptly brought under control by rigid prophylaxis. It should have equal value in protecting the members of public health teams required to attend cholera patients.

CONCLUSIONS

Phthalylsulfacetimide is distinguished from other sulfonamides by its low toxicity, its marked action against the cholera vibrio, and its unique property of

being concentrated in the intestinal wall proper. The compound was administered to forty patients with cholera, of whom thirty-nine survived. This experience compares favorably with those obtained with the use of sulfonamides in conjunction with saline, although detailed comparisons are not warranted because of the uncertain validity of statistics thus obtained. No evidence of toxic effect of any kind whatever, attributable to the new drug, was observed. It is felt that the data presented establish an important role for phthalylsulfacetimide in the treatment of cholera. While that aspect is paramount, the epidemiological importance of clearing *V. comma* from the stools, for the purpose of mass prophylaxis, also deserves great emphasis.

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19. SENECA, H., AND HENDERSON, E.: Action of Phthalylsulfacetimide on Certain Bacteria. To Be Published.

CORRESPONDENCE

February 3, 1949

Dr. Mark F. Boyd, Editor
American Journal of Tropical Medicine
615 East Sixth Avenue,
Tallahassee, Florida.

Dear Dr. Boyd:

I wish to disagree with the Article "The Teaching of Malaria Diagnosis" by A. J. Walker.

It is just as bad to insist on teaching only thick film method as it would be to teach only thin films. Both should be thoroughly taught. Thick films do increase positive findings and so must be taught; but one who relies on this method alone is like the man who would base pathologic diagnosis on gross appearances alone, neglecting microscopic details. The fact that once this was all anyone had is immaterial.

If one wishes to study the life history of the parasites and their real structure and differences, as well as determining if there are sufficient gametocytes to infect mosquitoes, well stained thin films are an essential. It will take on the average, three laboratory periods of three hours each to teach this. This conclusion is based on over twenty years of teaching.

Yours sincerely,

EDWARD B. VEDDER

BOOKS RECEIVED

- A. C. ROXBURGH. *Common Skin Diseases*. 8th ed. pp. xxi and 497, figs. 212 with 8 color plates. The Blakiston Co., Philadelphia, Penna. 1949. \$7.00.
- NAUCK, E. G., ENIGK, K., REICHENOW, E., VOGEL, J., WESTPHAL, A., AND WEYER, F. *Tropenmedizin und Parasitologie*. Fiat Review of German Science, 1939-1946, pp. 253. Fiat (Field Information Agency Technical, U. S.) Office of Military Government for Germany. Wiesbaden, Germany. 1948.
- THOMAS M. RIVERS, Editor, with twenty-six associates. *Viral and Rickettsial Infections of Man*. Pp. 544, figs. 77 with 6 color plates. J. B. Lippincott Co., Philadelphia, Penna. 1949. \$5.00.
- RICHARD L. SUTTON AND RICHARD L. SUTTON, JR. *Handbook of Diseases of the Skin*. Pp. xiii and 749, figs. 1057. The C. V. Mosby Co., St. Louis, Mo. 1949. \$12.50.
- GONZALO PIEDROLA GIL. *Recientes Adquisiciones y Tecnicas de Empleo del D.D.T.* Prólogo del V. Matilla. pp. vii and 307, figs. 54. Publicaciones del Instituto Español de Medicina Colonial. Consejo Superior de Investigaciones Científicas. Madrid, Spain, 1948. Wrap. 110 ptas.

BOOK REVIEWS

THOMAS M. RIVERS, Editor. *Viral and Rickettsial Infections of Man*. Pp. 544, Figs. 77 with 6 color plates. J. B. Lippincott Company, Philadelphia, Penna., 1949. \$5.00.

This new and very complete work, now in the first edition, is recently off the press. The chief subjects dealt with are: General Aspects of Viral and Rickettsial Infections, authored by the editor; Physical and Chemical Procedures, by W. M. Stanley and Max A. Lauffer; Serologic Reactions in Viral and Rickettsial Infections, by Joseph E. Smadel; Chick Embryo Techniques, by E. W. Goodpasture and G. John Buddingh; Propagation of Viruses and Rickettsiae in Tissue Cultures, by John F. Enders; Epidemiology, by Kenneth F. Maxey; and Bacterial Viruses (Bacteriophages), by A. D. Hershey and J. Bronfenbrenner. Also, there are separate chapters dealing with specific viral and rickettsial diseases, including many colored illustrations, which are excellent. These chapters include complete descriptions of all important virus and rickettsial diseases with sufficient bibliography to make additional references readily available. Since there are a total of thirty-seven chapters, it has been impossible in the scope of this review to deal with each separately; therefore, those dealing with separate diseases have been treated as a whole.

The text is one that the medical profession has long needed, and which, if improved by including more detailed and exact technique in later editions, will prove to be of great value to the laboratory worker. The present edition, although obviously written for the medical student and practitioner, should serve as a useful guide to the laboratory worker in virology. As a mark of its usefulness, since the arrival of the book in this laboratory, it has been in constant use, and the demand has so greatly exceeded availability that a number of personal copies have been ordered.

The first part of the book is concerned with techniques, and although this field changes constantly, it forms a good basis upon which search of the literature for new developments may be added. At the same time one of the outstanding values of the work to the non-laboratory worker is that it does not grow elaborate in the description of techniques, but does describe methods of collection, preservation and transportation of specimens. Each technique and each disease are described by an author thoroughly experienced and competent in the field and herein lies one of the outstanding values of this compendium.

This work would provide a good text in virology for the student as well as an excellent source for the instruction in the subjects treated. The last chapter, dealing with the subject of encephalomyocarditis was originally observed by this reviewer in two American soldiers at the Clark Field Station Hospital on Luzon, P. I., while laboratory consultant for the Southwest Pacific Theatre in 1946. Much credit is due Dr. Joel Warren and Dr. Joseph E. Smadel of the Army Medical Research and Graduate School for working out the etiology of this disease, then termed by us, "Three Day Fever."

A last superficial point but not the least important is the exceptional quality of paper, binding, and printing at the very moderate cost of \$5.00. This was made possible by the partial financing of its production by the National Poliomyelitis Foundation.

DWIGHT M. KUHN

NAUCK, E. G., ENIGK, K., REICHENOW, E., VOGEL, J., WESTPHAL, A., AND WEYER, F. *Tropenmedizin und Parasitologie*. Fiat Review of German Science, 1939-1946, 253 pp. Fiat (Field Information Agency Technical, U. S.), Office of Military Government for Germany, Wiesbaden, Germany, 1948.

This digest of German contributions in the associated fields of tropical medicine and parasitology covers the period of the war years when much of German science was not available to the Allied Nations. The book is divided into four sections: I. General Tropical Hygiene, by Doctor Nauck, presently director of the Hamburg Tropical Institute; II. Parasitology, by Doctors Reichenow and Weyer; III. Tropical Diseases of Man, by Doctors Nauck, Reichenow, Vogel, Westphal and Weyer, and IV. Tropical Diseases of Domestic Animals, by Dr. Enigk.

In the first section there is a concise but comprehensive report on the German literature in the field of tropical medicine, including texts, handbooks and collected works, journals, and many research papers. There are brief chapters reviewing tropical medicine and hygiene in the war; colonial sanitary service; general observations on tropical diseases and their dissemination; living conditions, clothing and nutritional requirements in warm climates, and climatic hardships and acclimatization in the tropics. The review constitutes a condensation of the contributions found in 254 separate publications.

The section on parasitology is confined to protozoölogy, by Reichenow, and entomology, by Weyer. Under brief chapters on the intestinal protozoa and related species, trypanosomes, coccidia, hemosporidia (with special emphasis on exoerythrocytic stages of malaria parasites) and the cnidosporidia, the work of 78 investigators is correlated. The chapter on entomology, based on only 17 references, consists merely of a listing of authors and the subjects of their publications.

The third section, like the first, is well organized, well developed and abundantly documented. It comprises the following chapters: malaria and blackwater fever (187 references); leishmaniasis and trypanosomiasis (48 references); tropical dysentery, with consideration of amebiasis, balantidiasis, coccidiosis, flagellate infections of the large bowel, blastocystis infection and giardiasis (48 references); spirochetosis, including relapsing fever and frambesia (14 references); bacterial diseases, consisting of brucellosis, cholera, leprosy and plague but omitting shigellosis (128 references); the viral diseases, papataci fever and yellow fever, and the tropical rickettsioses (45 references); tropical helminthiasis with special consideration of schistosomiasis, paragonimiasis, hookworm disease, strongyloidiasis and filariasis (52 references); tropical mycology and dermatology (19 references); nutritional diseases, especially beriberi, pellagra and sprue (35 references), and venomous animals and their venoms (31 references).

The fourth section covers approximately the same etiological fields for domestic animals as the third section includes for human infections, and, in addition, the diseases caused by arthropods, and hygiene and nutrition. There are 354 literature citations.

With the exception of the chapter on medical entomology in section two, the compendium constitutes a well-balanced and relatively authoritative synthesis of the German literature in tropical medicine for the period covered. Not all of the original publications cited were new contributions to science but some of them were substantial additions to knowledge. Perhaps the single item which intrigued the reviewer most was the statement on page 30 that the Panzer Army in North Africa was well cared for from a nutritional standpoint, not only with the necessary caloric and vitamin essentials but in preparation for life in a foreign environment with a special field cookbook for warm countries.

In some respects the summaries presented in this book are comparable to the reviews of abstracts published frequently in *Tropical Diseases Bulletin*, except in the latter periodical the summaries are based on all of the important contributions to a particular subject. Actually many of the references cited in the German publication have been more fully abstracted in *Tropical Diseases Bulletin*. Nevertheless, a distinct service has been rendered by Doctor Nauck and his colleagues in preparing their volume.

E. C. FAUST

SUSCEPTIBILITY OF THE GUINEA PIG TO *ENDAMOEBIA HISTOLYTICA* OF HUMAN ORIGIN.¹

GUILLERMO M. CARRERA AND ERNEST CARROLL FAUST^{2,3}

INTRODUCTION

Several species of laboratory animals have been employed for the experimental study of amebiasis, particularly that resulting from inoculation with *Endamoeba histolytica* obtained from human sources. The degree of success attending such study has depended in part on the intrinsic susceptibility of the animal utilized, its age and its nutritional status, in part on the inoculation technic employed, and probably also in part on the pathogenicity of the particular strain of *E. histolytica* employed. Monkeys, dogs, kittens and rats have served as the more common experimental hosts, while young pigs and rabbits have occasionally been used. In the monkey the experimental infection is almost invariably mild and chronic. In the dog it varies in its severity, its acuteness or chronicity, depending on the age of the animal, its nutrition and the way in which it is pre-conditioned for the inoculum. The kitten has been found useful primarily for acute tests in determining the relative pathogenicity of different strains of *E. histolytica* and usually does not survive the acute stage of the infection. The rat is also of particular value in acute experiments; but in contrast to amebiasis in the kitten, the infection in the rat characteristically is spontaneously cleared before there is opportunity for it to become chronic.

Previous to the study undertaken by the present workers there were apparently only three attempts to infect the guinea-pig with *Endamoeba histolytica*. Werner (1908) states that consistently negative results were obtained in his efforts to produce intestinal infection in several guinea-pigs, as well as in his introduction of dysenteric stool material into the guinea-pig's liver. Baetjer and Sellards (1914) produced acute, fatal infection in three of four guinea-pigs inoculated with a dysenteric menstruum containing *E. histolytica*, while Chatton (1917) obtained two positives in six inoculated animals. The following year Chatton (1918) provided an accurate detailed description of the amebic lesions in his infected animals.

STATEMENT OF THE PROBLEM

The present study constitutes an inquiry into the susceptibility of the guinea-pig to infection with *Endamoeba histolytica* of human origin, together with the type and extent of the amebic lesion produced in this animal.

¹ Supported by a Research Grant-in-Aid, National Institutes of Health, Bethesda, Maryland and by a research grant of Eli Lilly and Co.

² With the assistance of Frances Willard Fuller and Helen Sherrard.

³ Department of Tropical Medicine and Public Health, Tulane University, New Orleans, La.

THE NATURAL PARASITES OF THE GUINEA-PIG

The accompanying table (Table 1) lists the infections which have previously been reported from the intestine of the guinea-pig. In the case of guinea-pigs from Brazil and Peru the host species are indicated, otherwise the hosts appear to be laboratory-bred. A study of the intestinal protozoa of the guinea-pig has been made by one of us and is being published (Faust, 1949).

MATERIALS AND METHODS

The experimental animal. The guinea-pigs used in the present study were obtained from various local supply houses and from stock bred in our own laboratory. No genetic history was available. Both sexes were represented in our series, with predominance of females. The animals were of various sizes, ranging in weight from 180 to 550 gm. All were maintained on Rockland C Fortified Guinea-pig Diet throughout our experiment, and each animal was kept in an individual cage from the day before inoculation until it was sacrificed. Thirty-five animals were employed in the experimental series and six others were used as controls.

Anatomically, the guinea-pig is well adapted for the type of experiment undertaken in the present study. The terminal ileum has a constant landmark which serves to identify it and to orient the observer in the cecal area. This landmark consists of a very prominent lymphoid patch located about 3 cm. from the ileocecal junction. The cecum is large and pouch-like, and there is no vermiform appendix. The colon diminishes in diameter fairly abruptly beyond the cecal pouch.

Inoculum. The single strain of *Endamoeba histolytica* utilized during this experiment was isolated on September 8, 1947, from a human case of amebic brain abscess (culture kindly provided by Dr. J. C. Swartzwelder, Louisiana State Medical Center). The amebae were maintained in culture by serial passages every 48-72 hours in Balamuth's and in L.E.S. medium. The material used for inoculation consisted of amebae grown for 24-48 hours in one of the above-mentioned media, pooled and concentrated by centrifugation (5' minutes at 1500 r.p.m.). An estimate of the number of amebae utilized in each inoculation was made by counting a representative sample in a hemocytometer chamber. The volume of inoculum varied between 2.5 and 7 cc. and the number of amebae per inoculum between 200,000 and 7,150,000. The bacterial flora which accompanies this strain of *E. histolytica* consists of 5 aerobic non-motile, gram-negative rods, provisionally allocated to the alcaligenes group, and a gram-positive micrococcus.

Description of experimental procedure. The guinea-pigs were tagged and epilated the day before inoculation. Epilation of the abdomen was accomplished with the depilatory described by Pitesky and Last (1948). No food was given during the day before inoculation but water was allowed. On the day of inoculation the weight of each animal was recorded. Inoculation was accomplished during laparotomy. The guinea-pigs were anesthetized with nembutal, 1 mg. per 40 gm. of body weight, administered intraperitoneally and supplemented by open ether

TABLE 1

*Intestinal Parasites Described From the Guinea-Pig (exclusive of experimental infections)**II. Protozoa*

- Class: RHIZOPODA, Family: Amoebidae
Endamoeba cobyac (Walker, 1908)—cecum (= *E. caviae* Chatton, 1918)
Endolimax caviae Hegner, 1926—cecum
- Class: MASTIGOPHORA
- Family: Chilomastigidae
Chilomastix intestinalis Kuczynski, 1914—cecum
- Family: Trichomonadidae
Trichomonas caviae Davaine, 1875—cecum
T. flagelliphora Faust, 1921—cecum
Eutrichomastix caviae (Grassi, 1881)—cecum
- Family: Cercomonadidae
Entromonas caviae Lynch, 1922—cecum
- Family: Embadomonadidae
Embadomonas intestinalis (Wenyon and O'Connor, 1917)—cecum
- Family: Monadidae
Chilomitus coviace da Fonseca, 1916—cecum, *Cavia aperea* and *C. porcella*, Brazil
Oikomonas termo (Ehrenberg, 1838)—feces
Selenomonas palpitans Simons, 1921—cecum
S. ruminantium (Certes, 1889)—cecum
Sphaeromonas communis Liebetanz, 1910—intestine, *Cavia porcella*, Brazil
S. rossica Yakimoff, Wassilewsky, Korniloff and Zuietkoff, 1921—feces
Globomonas parasitica da Fonseca, 1923
- Class: CILIATA
- Subclass: Holotricha
- | | |
|--|---|
| <i>Cyathodinium conicum</i> da Cunha, 1914 | } —cecum, <i>Cavia aperea</i> and <i>C. porcella</i> , Brazil |
| <i>C. piriforme</i> da Cunha, 1914 | |
| <i>C. vesiculosum</i> da Cunha, 1914 | |
| <i>Enterophrya elongata</i> Hasselmann, 1918 | } —cecum, <i>Cavia, aperea</i> , Brazil |
| <i>E. piriforme</i> Hasselmann, 1918 | |
- Subclass: Heterotricha
Balantidium caviae Neiva, da Cunha and Travassos, 1917—cecum, *Cavia aperea* Brazil
- Subclass: Oligotricha
Cunhaia curvata Hasselmann, 1918—cecum, *Cavia aperea*, Brazil
- Class: SPOROZOA
- Subclass: Coccidiomorpha
- Order: Coccidiida
Eimeria caviae Sheather, 1924—intestine (and liver?)

*II. Spirochaeta**Cristospirella caviae* Hollande, 1921—small intestine*III. Helminths*

Phylum: PLATYHELMINTHES

Class: TREMATODA

Fasciola hepatica Linnaeus, 1758—bile ducts

TABLE 1—Continued

Phylum: PLATYHELMINTHES—Continued

Class: CESTOIDEA

Hymenolepis nana var. *fraterna* (von Siebold, 1852) Stiles, 1906—small intestine

Taenia pisiformis (Bloch, 1780) Gmelin, 1790—cysticercus stage in *Cavia cutleri*, Peru

Phylum: NEMATODA

Ancylostoma caninum (Ercolani, 1859) Hall, 1913—small intestine

Cephalobus aberrans Steiner, 1929—feces

Paraspidodera uncinata (Rudolphi, 1819) Travassos, 1914—cecum, colon

Subulura uncinata (Rudolphi, 1819) Hall, 1916—cecum, *Cavia aperea*

Trichinella spiralis (Owen, 1835) Railliet, 1895—small intestine

inhalation as needed. Aseptic technic was used throughout the operative procedure. The abdomen was opened by a longitudinal midline incision 3 cm. in length, the terminal ileum was identified and exteriorized and the inoculum was slowly injected into the intestinal lumen between the terminal lymphoid patch and the ileocecal junction. A 20 g. hypodermic needle was used for the injection. The abdominal incision was closed in layers with cotton thread suture.

No food was given for 24 hours after the operation but the regular diet was resumed thereafter. Animals which died during the experiment were not included in the series except for one animal in the 5-day incubation group, which had died only a few hours previously. The inoculated guinea-pigs were sacrificed at scheduled intervals, using an overdose of nembutal.

Autopsies were performed immediately thereafter. The body weight was first recorded. Then the abdominal cavity was opened and grossly inspected, and the colon with attached segment of the ileum was dissected and removed to a flat tray for further study. As extended on the tray (see Figure 1), this portion of the bowel was arbitrarily designated as consisting of six successive levels, designated as follows: A (terminal ileum), I (cecum), II (ascending colon), III (transverse colon), IV (descending colon) and V (rectum). These segments with their fecal contents were then carefully separated from one another. Feces from each level were removed for parasitologic examination and for pH determinations, and representative portions of the wall of each level with adherent feces were fixed in Bouin's fluid for histologic study. The liver was removed with clean instruments, weighed, examined, sectioned and portions fixed in Bouin's fluid. All microscopic sections were stained with hematoxylin and eosin.

In the earlier part of this study, both before and following inoculation of the guinea-pig with *Endamoeba histolytica*, microscopic examination of the freshly passed fecal pellets was made daily in an attempt to discover the natural parasites in the intestinal tract of the particular animal and to detect the progress of the amebic infection. It was soon found that the feces contained only sporadic evidence of infection, and that the findings constituted no dependable index of the species or amount of parasites present as determined at necropsy. The only parasites discovered by ante-mortem examination were *Balantidium caviae*,

Chilomastix intestinalis and *Eimeria caviae*, the cysts of which were occasionally passed in the feces. Examination of passed feces was thereafter abandoned.

Parasitologic study at autopsy consisted in the removal of a representative sample of lumen feces to microscopic slides for immediate examination. Two 22 mm. square cover-glass preparations, one in physiologic saline and one in D'Antoni's iodine, were mounted from each sample and were carefully studied. Likewise, smear preparations were examined and cultures of liver material

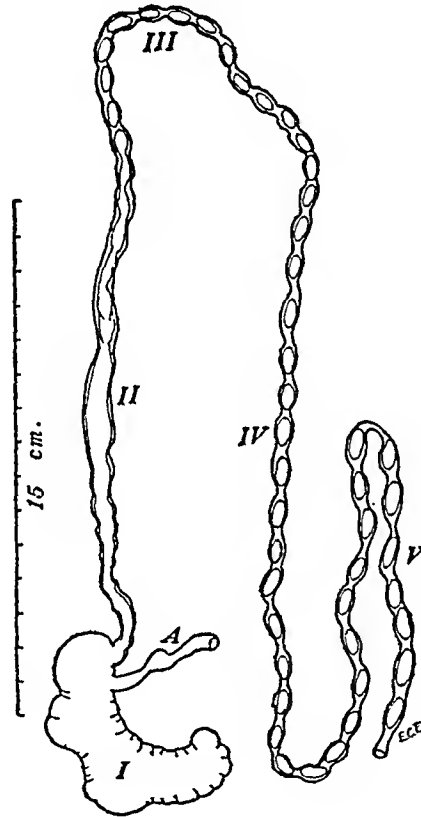


FIG. 1. Diagram showing the different intestinal levels of the guinea-pig involved in experimental amebiasis reported in this study. A, terminal ileum; I, cecum; II, ascending colon; III, transverse colon; IV, descending colon, and V, rectum.

were made to detect possible amebic invasion of this organ. In most instances cecal feces were also cultured for amebae.

RESULTS

Parasitological examination of intestinal contents

A. *The natural parasites of the guinea-pig's intestine.* In the six levels of the bowel from which lumen feces were examined from the 41 guinea-pigs in the series (six controls and 35 inoculated animals) the following parasites were identified: *Endamoeba cobayae*, twice, (once on the basis of several 8-nucleated cysts of this ameba in material from the ascending colon and once from culture of

cecal feces); *Balantidium caviae*, 24 times; *Spiromonas angusta*, 27 times; *Chilomastix intestinalis*, 16 times; *Trichomonas caviae*, 13 times; a *Bodo*-like flagellate, twice; *Eimeria caviae*, twice, and the oxyuroid nematode, *Paraspidodera uncinata*, once. Six animals of the series appeared to be free of natural infections, but this can be interpreted only to mean that the organisms were either not present or too few to be detected in the samplings.

Almost without exception the protozoa observed were present in greatest numbers in the cecal feces, and, with one exception, only in the trophozoite stage at this level. Their presence in the terminal ileum suggested that this was possibly due to a regurgitation of the cecal material. In the controls and in the majority of those experimentally inoculated with *E. histolytica*, the feces tended to form into fecal pellets in the lower part of the ascending colon, and from this level downwards to the anus dehydration of the feces was rapid. Except for *Spiromonas angusta* this dehydration was accompanied by a marked decrease in the number of trophozoites and a tendency to encystation, although the number of cysts observed was scant, even in the case of *Balantidium* and *Chilomastix*, both of which produce a thick, resistant cyst wall. Frequently in the dehydrating feces there was evidence of dwarfed trophozoites which were relatively inactive and showed internal degenerative changes. In contrast to this general situation, *Spiromonas angusta*, which has apparently not been reported previously from the guinea-pig, was present in the active trophozoite state in the feces as long as there was any appreciable moisture in the fecal pellets. Moreover, we were never able to identify the cyst of this flagellate in any of our material. *Eimeria caviae* was diagnosed twice from the intestinal contents, each time from a few oöcysts in the cecal and colonic feces. There was no clue as to the level of the intestine where the active tissue infection occurred.

The single helminthic infection consisted in eight mature specimens of the oxyuroid nematode, *Paraspidodera uncinata*, found free in the lumen of the cecum.

An analysis of the findings of these natural parasites of the guinea-pig by days of incubation following inoculation with *E. histolytica* suggested that they were just as likely to be present following luxuriant growth of this ameba as in the control series. However, the number of the flagellates and of the *Balantidium* was at times scant in the presence of a fulminating amebiasis.

None of the monadid flagellates or of the holotrichous and oligotrichous ciliates described for wild caviae in Brazil, and occasionally in domestic stock in other geographical locations, have been seen in our series of animals.

B. *Endamoeba histolytica* in the intestinal contents of the inoculated guinea-pig. In the six control animals there was no evidence of *E. histolytica* or any other ameba.

In four of the five animals which were sacrificed 24 hours after inoculation *E. histolytica* was demonstrated in lumen feces, always in small numbers and in the trophozoite state. In one animal the amebae were recovered from levels A, I and II, in another from I, II and III, and in the other two positives only from the cecal feces. Among the 5 animals sacrificed two days after inoculation only one provided evidence of infection from examination of the lumen feces, in

this instance at the cecal level. Among the 5 animals sacrificed on the third day all showed evidence of infection, one only in material from level A, two only from level I, one only from level II, and one from levels I-III.

Four days following inoculation 5 of 5 inoculated animals had positive lumen contents. In two the contents of levels A, I, II, III and IV contained active *E. histolytica* trophozoites; in a third, at A and I; in a fourth, at I and II, and in the fifth, only at level I. In all of these infections there were considerable mucus, red blood corpuscles and necrotic epithelial cells in a thin, gruelly, fecal menstruum.

All 5 of the 5 animals sacrificed on the fifth day were positive. In one animal the amebae were demonstrated from the feces of levels A, I, II and III; in two others from levels A, I, and II; in another from I, II and III (level A inadvertently not examined); and in the fifth, from levels I and II. Four of the five animals

TABLE 2

Summary of Findings of Endamoeba Histolytica In Lumen of Guinea Pigs' Intestine (and Liver) Following Experimental Intra-ileal Inoculation

TIME SACRIFICED POST-INOCULATION	NO. IN SERIES AND NO. +	NO. POSITIVE AT DIFFERENT LEVELS						
		A	I	II	III	IV	V	Liver
Controls	6 : 0	0	0	0	0	0	0	
1 day	5 : 4	1	4	2	1	0	0	
2 days	5 : 1	0	1	0	0	0	0	
3 days	5 : 5	1	3	2	1	0	0	
4 days	5 : 5	3	5	3	2	2	0	
5 days	5 : 5	3	5	5	2	0	0	
6 days	5 : 4	1	4	3	3	1	1	
7 days	5 : 5	1	5	2	1	1	0	1 (culture)
1-3 days	15 : 10	2	8	4	2	0	0	
4-7 days	20 : 19	8	19	13	8	4	1	
1-7 days	35 : 29	10	27	17	10	4	1	1 (culture)

sacrificed on the sixth day had demonstrable *E. histolytica* in the lumen contents, one at all levels A through V, two at levels I-III, and one only at level I. All of the 5 animals sacrificed on the seventh day provided evidence of *E. histolytica* in the intestinal lumen, one at levels A-II, one at levels I-IV, and three only at level I.

The findings are summarized in the accompanying table (Table 2).

It will be noted that during the first two days following inoculation the amebae present in the lumen contents were relatively sparse; yet some multiplication of the inoculum must have occurred, otherwise there would have been slight chance of finding any organisms in so large an amount of feces, particularly in the lumen of the pouch-like cecum. Beginning with the third day and particularly evident from the fourth through the seventh day there was ample evidence of colonization of the amebae. Moreover, even without pathological study of the

lesions in the bowel wall, there was an indication that the amebae in the lumen contents had been discharged from the lesions, viz., excess mucus, with blood and cellular detritus in close association with the lumen amebae.

With a single exception the amebae recovered from the lumen feces were in the trophozoite stage. Usually they were large and active, occasionally with ingested red blood cells and once with numerous starch granules. At times in the animals which were sacrificed on the fifth to seventh day following inoculation there was considerable decomposing cellular detritus and blood-stained mucus in the contents of the cecum and ascending colon. Invariably the amebae found in this material were sluggish or moribund. The exception referred to above was in a 5-day sacrifice, in which a few precysts and a single uninucleate cyst of *E. histolytica* were found at the level of the ascending colon in a menstruum of semiformed feces, in the midst of myriads of active *E. histolytica* trophozoites.

TABLE 3

Summary of pH Determinations at Different Levels of the Bowel in E. histolytica—Infected and Control Guinea-Pigs

	INOCULATED ANIMALS	CONTROLS
Numbers of animals examined.....	12	6
Range of pH in area I.....	6.5 -8.5	6.1 -7.0
Average pH in area I.....	7.43	6.43
Range of pH in Area III.....	6.4 -7.6	5.8 -7.1
Average pH in Area III.....	6.96	6.38
Range of pH in Area V.....	5.4 -7.0	5.7 -6.7
Average pH in Area V.....	6.02	6.03

pH determination of the feces

Twelve inoculated animals and six controls were examined. The inoculated animals all harbored *E. histolytica* at the time of examination. The pH values obtained in both groups are summarized in Table 3.

Pathologic findings

Observations on weight and general behavior. Even though the number of animals in the series is too small to give a definite indication as to the degree of variation in body weight after inoculation with *E. histolytica*, it was observed that the guinea-pigs lost weight fairly consistently. The average weight loss for all the animals, between the date of inoculation and the date of autopsy, was 37 gm. This loss was apparent on direct inspection and palpation. The animals were anorectic, hypoactive, apathetic and their fur was less glossy and alive than that of the control animals.

Gross examination. For convenience, the animals have been grouped according to the number of days after inoculation when they were sacrificed, i.e., 1, 2, 3, etc. All animals in the first and second groups (10 of 10) showed areas of hyperemia of the cecum (area I) as seen through the serosa. These areas could not be identi-

fied as corresponding to ulcers of the mucosa when the intestine was opened but appeared to constitute regions of congestion of the intestinal wall. In addition, all of these animals showed evidence of peritoneal irritation, although no free pus was encountered in the abdominal cavity. The ascending colon (area II) also showed congestion and appeared inflamed in one of the ten animals, while all other levels were free of gross changes. The liver showed yellowish areas ranging in size from 1 to about 10 mm. across in 3 out of 5 animals sacrificed after one day, and in 2 out of 5 animals sacrificed on the second day.

On the third day after inoculation all 5 animals showed lesions of the cecum (area I) which were classified as moderately extensive. These lesions were recognizable from the serosal aspect as irregular areas of inflammation, ranging in size from 3 to 10 mm. in diameter and corresponding to well-defined mucosal

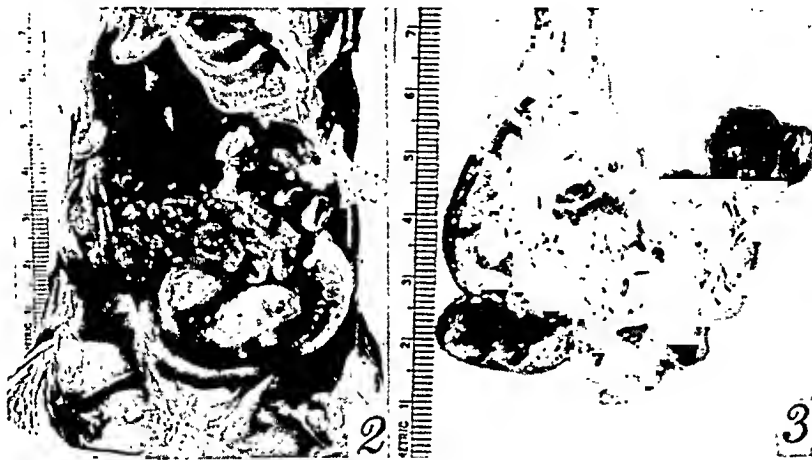


FIG. 2. Abdominal cavity of a guinea-pig sacrificed 7 days after inoculation. The cecum is the seat of extensive amebic invasion and as seen through the serosa the lesions appear as whitish to gray isolated and confluent zones of variable sizes. The rest of the gastrointestinal tract and the liver are normal.

FIG. 3. Internal aspect of the cecum of the same animal shown in fig. 2. There is extensive ulceration of the mucosa and thick whitish-gray pseudo-diphtheritic exudate over the ulcerated areas. A portion of the ascending colon shown in the upper part of the picture is free of lesions.

ulcers, covered with a whitish, mucoid, pseudodiphtheritic membrane. (Figures 2, 3.) The terminal ileum as well as all the other levels of the colon were macroscopically free of lesions. One of the 5 animals in group 3 showed hepatic lesions, consisting of small yellowish areas similar to those described in the animals of groups 1 and 2.

In group 4 all 5 animals inoculated showed definite gross lesions in area I, similar in appearance to, but more extensive than those observed in group 3. In this group, 4 of the 5 animals showed evidence of deep perforating ulceration, with walling off of the perforation by fairly firm fibrinous adhesions on the serosal aspect and binding the area of ulceration to nearby intestinal loops or to the parietal wall. Areas A, and II through V, were free of gross lesions. Two of the five animals showed hepatic lesions similar to those already described.

All animals sacrificed 5 days after inoculation revealed extensive ulceration of area I, similar in appearance to the lesions seen in groups 3 and 4. In one animal of this group there were inflammatory lesions in areas A and II which grossly resembled those of area I. The same animal, in addition to 3 others in the group, showed hepatic lesions similar to those already described. Area II was involved in another animal but not affected in the remaining 3. Areas III to V were unaffected in all animals of this group.

Four of the 5 animals in group 6 showed extensive ulceration of area I and one showed a lesion in area II. Area A was also inflamed in one animal. The other levels were free of lesions. The livers of 2 out of the 5 animals showed areas of necrosis.

TABLE 4

Summary of Macroscopic Findings at Autopsy of Guinea-Pigs in Experimental Amebiasis Study

+ = definite lesions; S = suggestive lesions

DAY AFTER INOCULATION	NO. IN SERIES	LEVEL OF BOWEL						
		A	I	II	III	IV	V	Liver
1	5	—	S(5)	S(1)	—	—	—	+(3)
2	5	—	+(1), S(4)	—	—	—	—	+(2)
3	5	—	+(5)	—	—	—	—	+(1)
4	5	—	+(5)	—	—	—	—	+(2)
5	4(5-1)*	+(1)	+(4)	+(1)	—	—	—	+(3)
6	5	—	+(4)	+(1)	—	—	—	+(2)
7	5	—	+(3), S(1)	—	—	—	—	+(4)
Controls	6	—	—	—	—	—	—	+(1)

No. of animals inoculated.....	35
No. died before sacrifice.....	1*
No. inoculated studied for lesions....	34 (100%)
No. with definite macroscopic lesions in bowel.....	22 (65%)
No. with suggestive macroscopic lesions in bowel.....	10 (29%)
No. with no macroscopic lesions in bowel.....	2 (6%)

The seventh day after inoculation 3 of the 5 animals showed definite lesions in area I, while one showed only a suggestive lesion, no true ulceration being grossly visible, although there was a focus of congestion of the cecal wall. Areas A and II to V were unaffected in all animals of this group. In 4 of the 5 animals the liver showed lesions similar to those already described.

The control animals revealed no intestinal lesions (areas A through V) and in only one of the 7 controls was there an hepatic lesion which grossly resembled those found in groups 1 to 7.

The gross pathologic findings are summarized in Table 4.

Histologic findings. During the acute phase of the infection there appears to be considerable similarity in the tissue reaction to infection with *E. histolytica* between the guinea-pig and that observed in the more common experimental

hosts, as well as in man. In general, it can be stated that invasion of the guinea-pig tissues with *E. histolytica* provokes an inflammation characterized principally by necrosis and lysis of tissue, and less consistently by leukocytic infiltration fibroblastic repair, and epithelial proliferation of the intestinal mucosa (Figs. 4, 5)

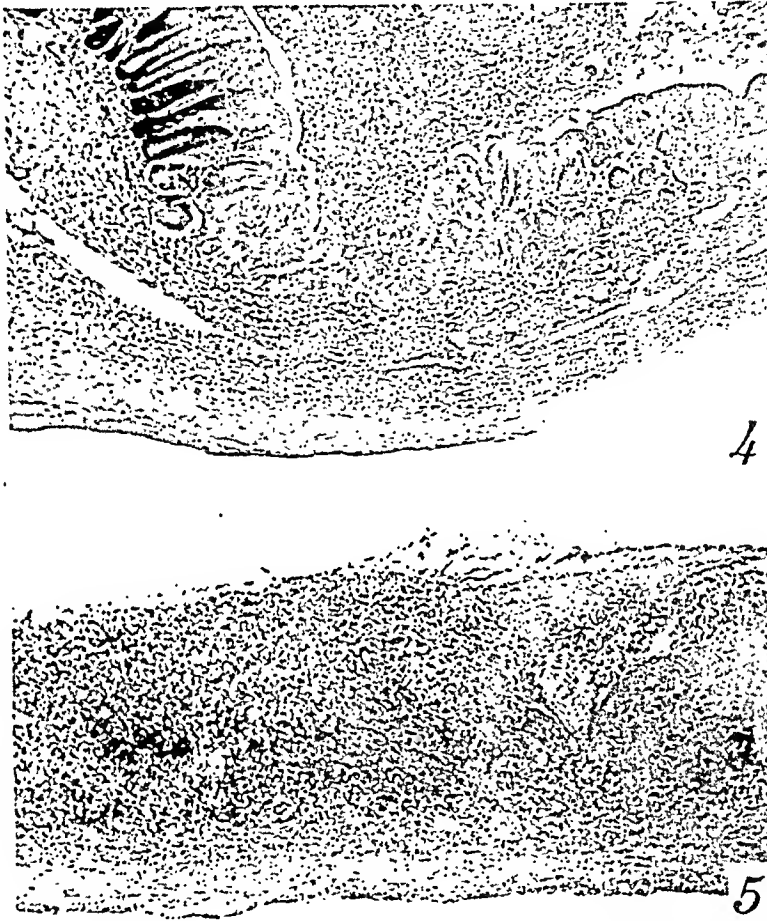


FIG. 4. Cecum of a guinea-pig sacrificed 3 days after inoculation. It shows a healing amebic lesion. The base of the ulcer has been covered by a single row of epithelial cells, but the crater still contains necrotic debris and amebae. Serial sections at this level demonstrated that the ulcer was still open at other levels and amebae were seen in the ulcer base and subadjacent tissues. The intestinal wall at this level shows a subacute type of inflammation. $\times 42$.

FIG. 5. Another level of the cecum of the same guinea-pig shown in fig. 4. There is complete destruction of the mucosa over a wide area and very dense acute and subacute inflammation of the submucosa, muscularis and serosal layers. Amebae were identified at various levels in this lesion. The surface of the ulcer is covered by necrotic material and leukocytes. $\times 42$.

Necrosis and lysis of tissue constituted perhaps the most striking change observed wherever the amebae were actively invading the host. The leukocytic infiltration, although prominent in most lesions, was not a constant finding. Nests and colonies of amebae were occasionally found in partially necrotic and lysed

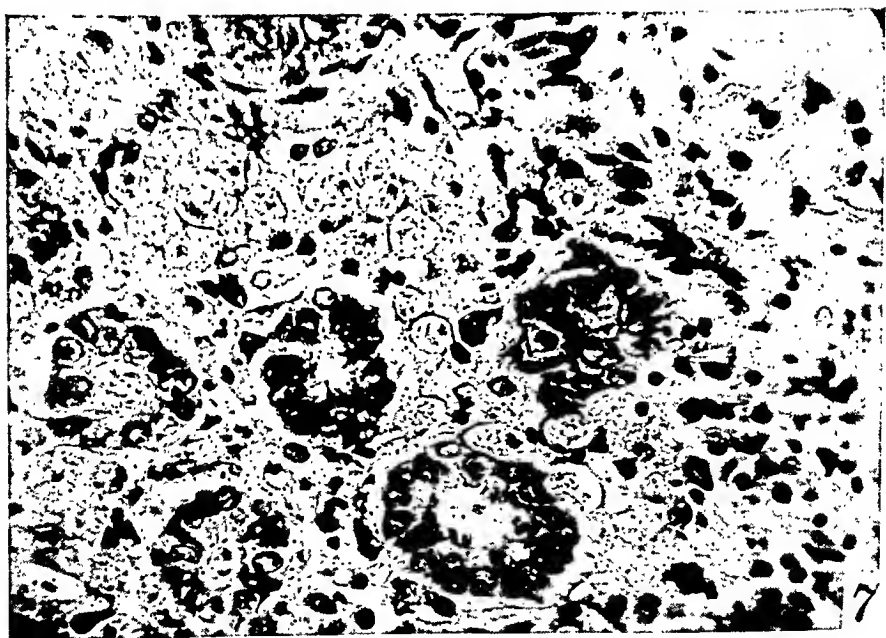


FIG. 6. Amebic lesion in the cecum of a guinea-pig sacrificed 2 days after inoculation. Many of the mucosal crypts have been invaded and replaced by colonies of amebae. In some areas there is no appreciable inflammation, in others there is minimal to moderate infiltration with lymphocytes and neutrophils. No areas of necrosis are evident, the amebae apparently having invaded the tissues by lysis in this uncomplicated type of lesion. $\times 128$.

FIG. 7. High-power magnification of the area outlined in black in figure 6. The amebae are clearly seen forming compact colonies replacing crypts and stroma. No leukocytic infiltration is evident in the regions of most notable amebic invasion. $\times 480$.

tissues, with practically no local leukocytic infiltration (Figs. 6, 7). The cells more commonly encountered in the areas of amebic invasion were polymorpho-

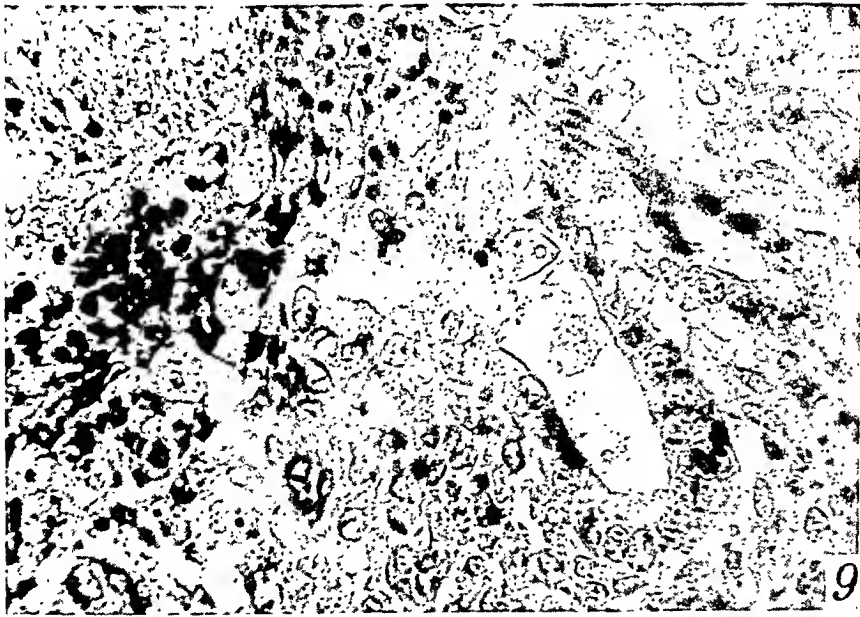


FIG. 8. Extensive mucosal and submucosal ulceration in the cecum of a guinea-pig on the fourth day after inoculation, with numerous amebae in some areas and thick necrotic exudate overlying the lesion. There is acute and subacute inflammation of the intestinal wall at this level. Amebae were identified in a micro-abscess of the subserosa at this level, in the region indicated by the arrows. $\times 128$.

FIG. 9. High power magnification of area outlined in figure 8. Note the amebae in and about a partially destroyed mucosal crypt, and the inflammatory exudate over it. $\times 480$.

nuclear neutrophils, lymphocytes, macrophages and plasma cells. Eosinophilic leukocytes were not observed in amebic lesions in the guinea-pigs, an observation which is in accord with what is known for other hosts with *E. histolytica*

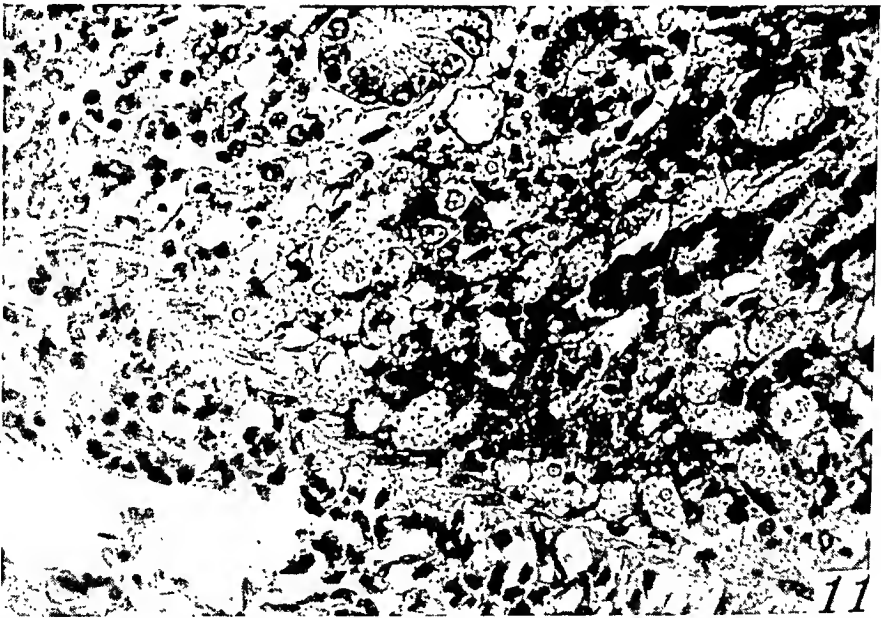


FIG. 10. Amebic invasion of the deeper portions of the mucosa of the guinea-pig's cecum 6 days after inoculation, without total necrosis of the superficial layers. There is moderate submucosal infiltration with neutrophils and lymphocytes. $\times 128$.

FIG. 11. High power detail of area outlined in fig. 10. Notice numerous amebae in the deeper layers of the mucosa as far as the muscularis mucosae, but not penetrating it. Many of the mucosal crypts have been destroyed and replaced by amebae. There is moderate inflammatory reaction of the mucosa and submucosa at this level. $\times 480$.

infection. Fibroblastic proliferation appeared early in most of the lesions and was usually prominent, not only in the direct neighborhood of the invaded area, but

also some distance away, where no amebae were found. The mucosal epithelial cells showed great activity at the borders of the lesions and mitotic figures were not uncommon in them.

On the first and second days after inoculation, sections of the intestine handled during the surgical procedure revealed a non-specific type of peritoneal inflammation characterized by fibrinous or fibrino-purulent reaction, edema and congestion. Moreover, on the mucosal aspect of the cecum during this very early phase of the infection there were foci of shallow erosion where *E. histolytica* was invading the glands and subepithelial tissues. These lesions were of irregular shape and did not go beyond the muscularis mucosae. In some instances extensive areas of very superficial invasion were seen, in others only small zones, not

TABLE 5

Summary of Microscopic Findings at Autopsy of Guinea-Pigs in Experimental Amebiasis Study

Eh = *E. histolytica* in lesion; + = amebic lesion without *E. histolytica*

DAY AFTER INOCULATION	NO. IN SERIES	LEVEL OF BOWEL						
		A	I	II	III	IV	V	Liver
1	5	—	Eh(4), +(1)	—	—	—	—	—
2	5	—	Eh(5)	—	—	—	—	—
3	5	—	Eh(5)	—	—	—	—	—
4	5	—	Eh(5)	—	Eh(1)	—	—	—
5	4(5-1)*	Eh(1)	Eh(4)	Eh(1)	—	—	—	—
6	5	—	Eh(5)	—	—	—	—	—
7	5	—	Eh(3), +(1)	—	—	—	—	Eh(1) culture
Controls	6	—	—	—	—	—	—	—

No. of animals inoculated.....	35
No. died before sacrifice.....	1*
No. inoculated studied for lesions.....	34(100%)
No. with amebic lesions in bowel.....	33(97%)
<i>E. histolytica</i> identified in lesions.....	31(91%)
Typical lesions but <i>E. histolytica</i> not seen.....	2(6%)
No. with no demonstrable lesions.....	1(3%)

extending beyond two or three crypts, were encountered. Rarely amebae were seen in the mucosa in regions directly covered by intact epithelium, but always in the neighborhood of open lesions.

By the third day after inoculation deep ulcers were encountered, some of which extended to the subserosa and even through the whole thickness of the intestine (Figs. 8, 9); yet no free perforations were observed, there being always, in the perforating type of ulcer, a walling-off process on the serosal aspect which prevented actual spilling into the peritoneal cavity. Fibroblastic and epithelial proliferation were at a minimum from the third day on, and partial covering of the ulcers by a single row of epithelial cells was often seen. In contrast to the excavated type of lesion observed in human beings with ulcerative amebic colitis

in which the lesions extend in all directions into the less compact submucosal layer rather than penetrating through the whole thickness of the intestine, the lesion in this acute phase in the guinea-pig is more commonly a superficial mucosal erosion or a deeply penetrating invasion, or both types simultaneously.

From the fourth through the seventh day after inoculation no important change in histologic appearance was observed in the lesions (Figs. 10, 11). Except in the regions of perforating ulcers there appeared to be less peritoneal inflammation, and there was evidence of very early superficial erosion as well as of extensive deep ulceration, similar in character to that described in the animals sacrificed 3 days after inoculation.

Table 5 summarizes the microscopic findings.

Observations on Balantidium and other protozoa. *Balantidium caviae* was observed in sections of 15 out of the 34 inoculated guinea-pigs and in 4 out of the 6 controls. The balantidia were usually found on the surface of the intestine or in the craters of amebic ulcers. In no instance were they seen invading the tissues more deeply than where the amebae were located. Those found in the amebic ulcers were frequently degenerate and rarely amebae were seen inside partially necrotic balantidia. In the uninoculated animals in which *B. caviae* was identified histologically there was no evidence of inflammatory reaction. With two exceptions the organisms were found in the lumen or on the surface of the intact mucosa. The exceptions consisted of a few sections in which an occasional balantidium could be seen under the mucosal epithelium, without any associated cellular reaction.

As regards the other intestinal protozoa of the guinea-pig, we found no evidence of pathogenicity; in the control animals the mucosal epithelium was intact and without any evident inflammation.

DISCUSSION

This report on the susceptibility of the guinea-pig to infection with *Endamoeba histolytica* of human origin provides considerable data on the development of the amebic lesion in a laboratory animal. Although the series was not large the results have been sufficiently uniform to answer a number of questions and to suggest profitable lines of future investigation.

The principal objective of this study has been achieved, namely, under the conditions of the experiment the guinea-pig has been found to be highly susceptible to infection with *Endamoeba histolytica*. In order to appreciate what this statement implies and not to overestimate its significance, a brief analysis will be made of the experiment as carried out and of the results achieved.

The experimental animal. Stock animals were used as they were available. They ranged in weight from 180 to 550 gm.; no difference was detected in their susceptibility to infection on a weight basis. However, animals weighing from 300–400 gm. are to be preferred, since smaller ones are poorer surgical risks and larger ones are more difficult to handle. There was likewise no evidence thus far that the sex of the experimental animal is a determining factor.

The food of the animals in the series was confined to a commercially prepared, fortified, guinea-pig dry ration, which was fed in generous amounts and was ade-

quately consumed. An analysis of this ration provided by the manufacturer indicates that it contains all of the essentials for full, balanced nutrition, but guinea-pigs not in this series, which had a supplement of greens, appeared to be somewhat more active and had a slightly glossier coat of fur. If such a supplement had been used by us in our experimental series, it is possible that the degree of susceptibility to *E. histolytica* infection might have been lower.

All animals in this series were starved for 24 hours preceding inoculation. This greatly reduced the content of the cecal pouch, so that when several cubic milliliters of inoculum were introduced into the terminal ileum, the inoculum was rapidly passed into the cecum and was not greatly diluted by cecal feces. We believe this to be a very important condition of the experiment, since it provided considerably greater opportunity for the amebae to make contact with the cecal mucosa and to initiate tissue invasion.

The experimental procedure of laparotomy and introduction of the inoculum were carried out under strictly aseptic technic. This required considerably more time than if the operation had been conducted without these precautions, but we feel more confidence in the results, since infection from faulty technic was practically eliminated. Moreover, the introduction of the inoculum into the terminal ileum, rather than through the wall of the cecum, required more time and more handling of the intestine, but there was no puncture of the cecal pouch which might have complicated interpretation of the gross or histologic picture at necropsy.

There was an abundance of normal fauna and flora of the guinea-pig's cecum. The fauna have been considered in the parasitologic portion of this report. The most conspicuous and constant elements in the flora were a large spirillum and a long, gram-negative bacillus. Comparison of the six uninoculated controls in our series with the inoculated animals has provided no evidence that any of the flora encountered or any of the fauna except *Balantidium caviae* and *Eimeria caviae* were tissue invaders, or that their presence necessarily provided particularly favorable circumstances for the development of the amebic lesion.

The inoculum. The inoculum consisted of *Endamoeba histolytica* obtained approximately a year and a half previously from an amebic lesion of the human brain. It had been grown in Balamuth's and LES medium, and on one occasion had produced an amebic liver abscess when introduced directly into the liver of an experimental animal. It was therefore known to have pathogenic capacity. Material from several culture tubes was pooled, so that 200,000 to 7,000,000 active amebic trophozoites were present in one to several cubic milliliters of inoculum. The associated bacteria consisted of five alcaligenes-type bacilli and one gram-positive micrococcus. This inoculum was never large enough in volume to cause appreciable distension of the cecal pouch, which was practically empty of feces at the time of inoculation.

During the first 48 hours following inoculation the cecal feces of animals which received large numbers of amebae contained more trophozoites than the cecal feces of those into which smaller numbers of amebae were introduced. However, from the third through the seventh day the richness of the inoculum had no detectable effect on the number of amebae in the cecal contents or on the extent

of the lesions in the intestinal wall. We feel that perhaps 20,000 to 50,000 active amebae, introduced under the conditions outlined above, should be sufficient to initiate invasion of the cecal wall within 24 to 48 hours after intra-ileal inoculation.

The parasitologic and pathologic findings. During the first three days following inoculation *E. histolytica* was found free in the cecal feces in 8 of the 15 animals, whereas typical amebic lesions were demonstrated in all 15 and *E. histolytica* was identified histologically in 14. During the remaining four days of the experiment the ameba was recovered from cecal and colonic feces in all but one animal. With a single exception all of these animals provided histologic evidence of tissue invasion by the amebae. Whereas the primary lesions were invariably at the cecal level and in only two animals were lesions verified microscopically at other levels, lumen trophozoites of *E. histolytica* were frequently found at various levels of the colon, at times in the terminal ileum and once in the rectum. Thus, during the first three days following inoculation there appears to be relatively little reliability of diagnosing tissue invasion on the basis of recovering lumen amebae. While the coincidence of lumen amebae and amebic lesions of the cecum was more striking from the fourth through the seventh day, the recovery of lumen amebae at levels below the cecum was not associated with histologically detected invasion of the wall at these levels. On the basis of these findings we have tentatively concluded that the amebae in the lumen of the cecum and colon during the first three days of incubation consisted primarily of residua or progeny of amebae in the inoculum which had not achieved tissue invasion, and to a lesser extent of the progeny of those amebae which had colonized in the wall (colonization demonstrated as soon as 24 hours after exposure). Thereafter the source of the lumen amebae was essentially, if not exclusively, the result of tissue colonization, since almost invariably these amebae were in a menstrium of cellular detritus and mucus, and occasionally had ingested red blood cells, even in the absence of gross hemorrhage.

It is of some interest to compare the macroscopic lesions (Table 4) with those demonstrated microscopically to be typically amebic in character (Table 5), even though the actual proof of amebic invasion of the bowel wall depends on the latter method of examination. In the group sacrificed 24 hours after inoculation, macroscopically there was suspicion of amebic invasion in 5 of 5 animals at the cecal level and in one of 5 in the ascending colon. Histologically there was confirmation for all 5 at the cecal level but no evidence in the ascending colon. In the 48-hour, 72-hour and 96-hour sacrifice groups (only cecal level lesions suspected), all 15 were confirmed histologically, but, in addition, *E. histolytica* was demonstrated once in a lesion in the transverse colon (96-hour group) which had not been grossly suspected. All of the macroscopically suggestive lesions in the 4 animals of the 5-day group which were subjected to microscopic tissue examination were demonstrated to have amebae in the lesions (viz., terminal ileum, 1 of 4, cecum, 4 of 4, and ascending colon, 1 of 4). In the 6-day sacrifice group there was macroscopic evidence of lesions at the cecal level in 4 of the 5 animals. By comparison, amebae were found histologically in the cecal wall in all 5, while the grossly suspected lesion in the ascending colon was not confirmed by study of the

sections. In the 7-day group 4 of the 5 animals had suspicious macroscopic lesions which were demonstrated histologically. In summary, careful microscopic study of the tissues confirmed all but two of the macroscopic diagnoses and added one lesion (transverse colon) which had been overlooked by gross examination.

In the guinea-pig, under the conditions of the experiment as planned and carried out, the amebae confined their invasion of the intestinal wall almost exclusively to the cecal level (33 of the 34 animals studied histologically), with additional lesions discovered only once each in the terminal ileum, ascending colon and transverse colon. Had the diet been of a type which favored liquid or semiliquid feces at lower levels of the colon, it seems possible that lesions might have been produced at these lower levels.

Architecturally the amebic lesions consisted of two principal types, the shallow, open cup-like ulcer and the more deeply penetrating one which is superficially much less conspicuous. Cytologically two distinct types of invasion were demonstrated. The first consisted of lytic necrosis, with essentially no host-tissue reaction and no evidence of bacterial invasion. This may be regarded as the uncomplicated amebic lesion. The second type was quite different: it presented evidence of bacterial invasion, with infiltration of polymorphonuclear neutrophils, lymphocytes, macrophages and plasma cells, and early fibroblastic proliferation, not only in the immediate vicinity of the amebic process but in adjacent zones in which no amebic invasion could be demonstrated. The histologic types of cecal amebiasis which we have found to develop in the guinea-pig correspond in all essentials to the amebic lesions at this level of the bowel in man and the dog, and to a certain extent in the monkey, kitten and rat.

Effect of amebic infection on the pH. Although the data summarized in Table 3 are too few for significance, they suggest that, at the levels affected by the invasion of the intestinal wall, the pH was definitely elevated (viz., 7.43 vs. 6.43 average). On the other hand, at the rectal level, essentially unaffected by the amebic infection, there was no change in the pH (viz., 6.02 vs. 6.03 average).

Effect of the infection on the health of the guinea-pig. Amebic infection produced loss in weight, anorexia, listlessness and deterioration in the appearance of the fur.

The hepatic lesion in cecal amebiasis. Macroscopic lesions were detected in 18 of 34 animals studied pathologically and in one control. In none of these animals was an amebic lesion demonstrated histologically, although in one animal of the 7-day group *E. histolytica* was recovered in culture of liver tissue from the site of a suspected lesion. It is concluded that the lesions observed were principally of other etiology.

Many questions have arisen as a result of our demonstration that the guinea-pig is highly susceptible to infection with *Endamoeba histolytica*. In the first place, although we have suggestive evidence that the normal fauna and flora of the guinea-pig play no essential rôle in the development of the amebic lesion in this animal, and have histologic evidence of uncomplicated lytic necrosis occurring at times around and in the midst of colonies of *E. histolytica* apparently free of bacteria and their characteristic host-cell reactions, as yet we have no proof that the bacterial associates introduced in the inoculum may not at times aid in the establishment of the type of lesion in which neutrophilic infiltration is conspicuous.

Nor do we know how readily the amebae could penetrate the mucosa if they were deprived of their bacterial associates.

A second type of inquiry is to be directed at the nutritional status of the host in relation to its susceptibility to amebiasis. Earlier in this discussion it was suggested that the diet of the guinea-pigs in this experiment was adequate for nutrition but without green food was apparently not optimum. It will be desirable to test this idea and likewise to determine in the guinea-pig what effect different types of deficient diets may have on susceptibility to infection with *E. histolytica*.

Another question is whether cysts of the same strain of *E. histolytica*, when fed by mouth or introduced by gastric tube, will produce infection in as high a percentage of guinea-pigs as when trophozoites are introduced into the terminal ileum.

Still another line of attack on the pathogenesis of *E. histolytica* in the guinea-pig consists in subjecting the animal to other strains of this species of ameba, to determine whether equally high infectivity can be demonstrated under the conditions of this experiment.

Finally, our experiment has been limited to the first seven days following inoculation. There is as yet no substantial evidence as to the course of the infection beyond the seventh day, either with respect to the survival of the host or of the parasite.

SUMMARY AND CONCLUSIONS

1. Only three previous attempts have been reported in which the guinea-pig has been exposed to infection with *Endamoeba histolytica*. Two of these (Baetjer and Sellards, 1914 and Chatton, 1917) were partially successful in producing tissue invasion and one (Werner, 1908) was unsuccessful.

2. The study herein reported consisted of a planned experiment to inoculate stock guinea-pigs under strictly aseptic technic with a strain of *Endamoeba histolytica* of human origin. Cultured trophozoites in numbers varying from 200,000 to 7,000,000 were introduced into the terminal ileum and the animals were sacrificed in groups of five at daily intervals from one to seven days following inoculation. Six uninoculated animals served as controls.

3. On sacrifice the terminal ileum and entire large intestine were removed intact, as was the liver. The several levels were grossly inspected for suspicious amebic lesions, then these levels, designated A (terminal ileum), I (cecum), II (ascending colon), III (transverse colon), IV (descending colon) and V (rectum), were separated from one another, pH determinations made on representative animals at levels I, III and V, the fecal contents carefully studied for evidences of *E. histolytica* and for the natural parasites of the guinea-pig's intestine, and segments of the intestinal wall at each level fixed in Bouin's fluid, sectioned and studied microscopically.

4. The natural parasites of the guinea-pig which were identified from cecal and colonic feces were: *Balantidium caviae*, *Chilomastix intestinalis*, *Trichomonas caviae*, *Spiromonas angusta*, a Bodo-like flagellate, *Eimeria caviae* and once the oxyuroid nematode *Paraspidodera uncinata*. Except for *B. caviae* and *Eimeria*

caviae, none of these organisms are tissue invaders. In no instance was there evidence that any of these organisms had assisted in the production of the amebic lesion. It was found that examination of passed fecal pellets was an unreliable method of diagnosing these infections.

5. *Endamoeba histolytica* was recovered from the intestinal feces in 8 of the 15 animals sacrificed on days 1-3 after inoculation, whereas typical amebic lesions were found in all 15. During days 4-7 the amebae were found in 19 of the 20 sacrificed animals and typical amebic lesions in 19, although the exceptions in each category were not coincidental. With two exceptions the lesions were confined to the cecal area; *E. histolytica* trophozoites were most frequently and abundantly recovered from this level but they were relatively common in the feces at levels A and II, occasionally at levels III and IV, and once at level V. The six control animals had the same natural fauna and flora found in the experimental series; in none of them were there any lesions of the intestinal wall in levels A and I-V.

6. The amebic lesions consisted of two types, (1) shallow excavations with a relatively wide diameter, and (2) deeply penetrating ones with a small orifice. Histologically some lesions consisted essentially of lytic necrosis without evidence of bacterial invasion and without host-cell reaction, and others with inflammatory reaction in which neutrophilic leukocytes and fibrocytes were predominant.

7. Amebic infection produced an appreciable increase in the pH at level I, slight increase at level III and no change at level V.

8. Spontaneous amebic lesions of the liver were found to be uncommon compared with lesions of undetermined origin observed in the guinea-pig's liver.

9. Amebiasis in the guinea-pig causes loss in weight, anorexia, reduced activity and deterioration in the appearance of the fur.

10. This study has conclusively demonstrated the high susceptibility of the guinea-pig to infection with a pathogenic strain of *Endamoeba histolytica* under the conditions of the experiment. It suggests the need to determine the rôle of the bacteria associated with the amebae in the inoculum, the relation of the nutritional status to susceptibility, the relative infectivity of cysts inoculated by mouth or intragastrically *vs.* trophozoites introduced into the terminal ileum, and possible differences in infectivity of other strains of *E. histolytica*.

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THE EFFECT OF OZONE IN WATER ON CYSTS OF *ENDAMOEBIA HISTOLYTICA*

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INTRODUCTION

Amoebiasis, under some circumstances at least, can be water-borne. Furthermore, numerous investigations have indicated that the chlorination procedures usually employed in the bacterial disinfection of water are relatively ineffective in destroying the cysts of the causative agent, *Endamoeba histolytica*. Consequently, the need for a disinfecting agent that would be cysticidal within practical limits of time and concentration and otherwise be suitable for use in water treatment has been generally recognized.

Interest in ozone as a cysticidal agent has been prompted by its value in water treatment, not only for taste and odor control but also as a bactericide. The one previous study on the effects of ozone on cysts, by Kessel *et al.* (1), indicated a high cysticidal efficacy. These results stimulated additional investigations in order to evaluate the cysticidal action of ozone under more varied conditions and with different experimental techniques.

MATERIALS AND METHODS

Ozone production. The ozone used in these experiments was obtained by passing chemically dried and ice-cooled oxygen through a 15,000-volt silent discharge.² This resulted in the production of an estimated 2 to 3 per cent ozone. The mixture of oxygen and ozone was then bubbled through a delivery tube with a diffuser into 1 to 3 liters of tap or distilled water in a 3-liter round-bottomed flask for various periods of time, depending upon the concentration of ozone desired. Excess gas was delivered to a solution of sodium iodide and boric acid to trap undissolved ozone.

In preliminary tests to ascertain a suitable method of obtaining aqueous solutions of ozone, it was found that factors such as the period of ozonization, pH, and the temperature determined the ultimate concentration of ozone. Increasing the ozonization period increased the ozone concentration to what might be designated as a saturation point. The temperature and, to a lesser extent, the pH appeared to determine this point. While this behavior is somewhat similar to that of the usual aqueous solution of gasses, it is probably complicated by the instability of ozone in water. It was noted that ozone concentrations decreased rapidly even in distilled water once ozonization stopped, presumably as the result of the decomposition of the gas. It was noted further that increases in

¹ Laboratory of Tropical Diseases, Microbiological Institute, National Institutes of Health, Bethesda, Maryland.

² We are indebted to Theodore D. Perrine, Laboratory of Chemistry and Chemotherapy, Experimental Biology and Medicine Institute, National Institutes of Health, for making available the ozone equipment used in these studies.

temperature increased the rate of decomposition. In the experiments reported here, the "applied" ozone concentrations tested for cysticidal action were usually lower than the obtainable saturation points.

Ozone determinations were made by titrating, with N/100 sodium thiosulphate solution, a 100-ml. sample to which were added sodium iodide and boric acid. In the ozone determinations for the earlier tests, in which water was used directly from the tap, allowances were made for the residual chlorine when it was present in measurable amounts. Prior to use in later tests, tap water was allowed to stand until the chlorine had disappeared.

Cyst material. Cysts of the NRS strain of *Endamoeba histolytica* were used throughout the experiments reported in this paper. Cysts in quantity were obtained in the following manner: Trophozoites maintained on rice-free whole egg slants overlaid with modified Locke's solution were transferred to similar media containing small quantities of rice flour. The resulting cultures were incubated at 37°C. for 3 days, after which time the cyst-containing sediment at the bottom of the slants was pooled, washed and sedimented by centrifugation in distilled water, strained through several thicknesses of gauze, and stored overnight in the refrigerator at approximately 5°C. Estimations of cyst yields were made after refrigeration and were based on counts of 0.05-ml. samples of diluted material. Despite straining, cyst batches contained a varying and sometimes considerable amount of extraneous material, for the most part small particles of the rice flour. The age of the cysts used in these experiments varied from 1 to 5 days; in most instances it was only 1 day.

Experimental procedure. Although individual experiments varied to some extent, in accordance with the demands of the particular variables under observation, a rather generalized procedure was usually followed:

1. The water, 1,000 to 3,000 mls. in volume, was ozonized for a period of time dependent upon the concentration of ozone desired.

2. A 100-ml. sample was poured off for the "applied" ozone determination.

3. A few mls. of the cyst inoculum were added to provide a concentration of 20 to 50, usually about 40, cysts per ml.

4. The flask was shaken vigorously and another sample poured off for determination of the immediate "ozone demand" of the cyst material.

5. At the desired time interval, a sample was poured off for the ozone determination. For cyst recovery, a larger sample, 500 to 750 mls., was poured into a beaker containing sufficient N/100 sodium thiosulphate to stop the action of the ozone.

6. The beakers containing cysts and water to a depth of 3 to 5 inches were left undisturbed for 4 to 5 hours, for the purpose of concentrating the cysts. At the end of this time counts indicated that the majority of the cysts had settled to the bottom. The supernatant fluid was then siphoned off.

7. Dilution counts were made of the cysts recovered in the sediment and samples containing various numbers were planted into tubes of the egg medium containing rice flour.

8. NRS bacteria that had been maintained in amoeba-free egg-slant cultures were added to the test cultures.

9. For each experiment, controls were planted with various numbers of untreated cysts that had been kept at the same temperature as the treated cysts. NRS bacteria were also added to the controls.

10. A sample of each culture was examined after incubation at 37°C. for 72 hours and, if negative, transfers were made to fresh medium and the transfers and original cultures examined 72 hours later.

Methods of evaluating cyst destruction. As indicated above, the criterion used for determining the viability of treated cysts was their ability to develop in culture. In the early experiments, large numbers of treated cysts were planted into each culture tube. The cysts were concentrated, divided into five equal portions, and each portion planted into a tube of medium. Each culture received 3,000 to 4,000 cysts. Failure to get positive cultures was interpreted as due to destruction of virtually all of the cysts. As controls for this series, several tubes were planted with approximately 2,000 untreated cysts per tube. For the purpose of later discussion this method of planting cysts is designated as type I.

It was apparent from the results of these experiments that a reasonable estimate of the amount of cyst destruction was not possible with the type I method of planting when positive cultures were obtained. With such large numbers of cysts, the survival of only a low percentage would result in positive cultures. Consequently, in subsequent experiments cysts were planted in decreasing numbers in a series of tubes rather than in large numbers only. If all cultures inoculated with the larger numbers of cysts were positive, the results obtained with smaller numbers would provide an indication of the amount of cyst survival. For example, a considerable amount of growth with the smaller numbers would indicate a relatively high amount of survival whereas little or no growth would indicate very little survival.

Accordingly, a second method of planting cysts, designated as type II, was employed in a number of experiments. Treated cysts were concentrated to 1,000 cysts per ml. and were usually planted into 25 tubes in numbers of 2,000, 1,000, 500, 250, and 125 cysts per tube, with 5 tubes in each group. Numbers of cysts were determined by dilution counts; consequently, the above figures represent average rather than exact numbers of cysts. The counts were made on several 0.05-ml. samples and the extremes of any series of counts usually were within 10 per cent of the average.

The untreated control cysts were similarly planted in a series of decreasing numbers, but with fewer cysts per tube, e.g., 30, 15, 8, and 4. This was done in order to determine the minimal average number of untreated cysts which, when planted into 5 culture tubes, resulted in 5 positives consistently. Such data would serve as a basis for evaluating the results obtained with any 5-tube group of cultures planted with treated cysts, in terms of the most likely number of viable cysts present.

In an attempt to find the most satisfactory way of evaluating the amount of

cyst destruction when it was less than complete, a third method of planting cysts, type III, was employed. The principle of decreasing numbers of cysts was retained, but the inocula were planted in logarithmically decreasing quantities in a manner similar to that employed in the determination of the most probable numbers of viable bacteria in water samples (2).

In experiments in which this type of planting was employed, treated cysts were concentrated to 1,000 cysts per ml. and portions of this suspension were planted into 15 tubes of medium. Five tubes each received 1 ml. (1,000 cysts), 5 received 0.1 ml. (100 cysts), and 5 tubes received 0.01 ml. (10 cysts per tube) of the cyst concentrate. Appropriate dilutions were made in order that all 15 tubes would receive the same quantity of fluid. Untreated control cysts for these experiments were planted in a series of decreasing numbers; 20, 10, and 5 cysts per tube.

The application of these three methods of planting cysts in the evaluation of cyst survival will be discussed along with the results.

TABLE 1

Growth of cysts of E. histolytica exposed to ozone in water and planted in concentrations of 3,000 to 4,000 per tube (temperature 20° to 24°C., pH 7.5 to 8.0)

EXPERIMENT NUMBER	5-MINUTE OZONE RESIDUALS (P.P.M.)	NUMBER POSITIVE OF 5 TUBES PLANTED	APPLIED OZONE (P.P.M.)
1	3.5	0	5.10
2	2.5	0	3.90
3	1.8	0	4.70
4	1.6	2	4.50
5	1.1	2	2.00
6	0.6	2	1.40
7	0.6	5	1.30
8	0.1	5	0.34

Variables evaluated. Of the variables other than concentration that might be expected to influence the effect of ozone, attention was given to temperature, pH, type of water, contact time, and organic nitrogen.³ No attempt was made to evaluate all of these variables over a wide range. Comparisons were made of temperatures of 10°, 19°, and 27°C.; of pH 6.5 with a pH ranging from 7.5 to 8.0; of tap water with distilled water; and of exposure periods of $\frac{1}{2}$, 1, 3, 5, and 10 minutes, to determine whether these variables exerted any marked effect upon the cysticidal action of ozone. Although no attempt was made to control organic nitrogen content, determinations were made routinely on the cyst-water mixtures, in order to provide some estimate of the extraneous matter present.

RESULTS AND DISCUSSION

The results of the early experiments on cysticidal ozone residuals are shown in Table 1. In this series, cysts were exposed for 5 minutes to various concentra-

³ We are indebted to the Microanalytical Service Laboratory, National Institutes of Health, for the organic nitrogen determinations.

tions of ozone. The type I method of planting cysts was employed. All 5 of the test cultures were negative when ozone residuals of 1.8 P.P.M. and above were used; below that concentration the ozone did not appear to be very effective, as evidenced by the occurrence of positive cultures. However, in experiments 4, 5, and 6, the same cultural results were obtained even though the residuals varied from 0.6 to 1.6 P.P.M. On the other hand, with the same ozone residuals, different numbers of positive cultures were found, as in experiments 6 and 7. These somewhat erratic results raised the question as to the amount of cyst survival represented by the positive cultures.

TABLE 2

Growth of untreated cysts used as controls for experiments on cysticidal 5-minute ozone residuals

EXPERIMENT NUMBER	NUMBER POSITIVES PER 5 CULTURES PLANTED WITH VARIOUS NUMBERS OF CYSTS			
	30	15	8	4
1	5	5	5	4
2	5	5	5	5
3	5	5	4	4
4	5	5	5	4
5	5	5	5	3
6	5	5	5	4
7	5	5	5	2
8	5	5	4	3
9	5	5	5	5
10	5	5	5	5
11	5	5	5	4
12	5	5	5	3
13	5	5	5	2
14	5	5	5	3
15	5	5	4	4
Total positives.....	75	75	72	55
Total cultures.....	75	75	75	75
Average number positives per 5 cultures.....	5.0	5.0	4.8	3.7

Such growth could have resulted from the survival of only a very small percentage of the several thousand cysts planted per tube.

In order to provide a means of estimating the amount of cyst survival when positive cultures occurred, the type II method of planting cysts was adopted. This method was used in 15 additional experiments to determine cysticidal 5-minute ozone residuals.

As a basis for evaluating the data for treated cysts, the results obtained with the untreated control cysts (Table 2) will be discussed first. It is apparent from this table that 5 positive cultures always resulted when 5 tubes were planted with an average of 15 or more untreated cysts per tube, and almost always developed when an average of 8 cysts was planted per tube. When an average of

4 cysts per tube was planted into 5 tubes, 3 or 4 positive cultures resulted in most cases.

From these data on untreated cysts, it was concluded that an average of 10 cysts per tube would result consistently in a positive culture.⁴ Therefore, when treated cysts were planted into 5 culture tubes, the failure to obtain 5 positive cultures would indicate the presence of less than 10 viable cysts per culture tube. Furthermore, some estimate of the probable number of viable cysts present could be made by a direct comparison of the growth of the treated cysts with that obtained for the untreated cysts shown in Table 2.

TABLE 3

Growth of cysts of E. histolytica exposed to ozone in water for 5 minutes and planted in varying concentrations per tube (temperature 19°C., pH 7.5 to 8.0)

EXPERIMENT NUMBER	5-MINUTE OZONE- RESIDUAL (P.P.M.)	NUMBER POSITIVE CULTURES OF 5 TUBES PLANTED WITH VARIOUS NUMBERS OF CYSTS					APPLIED OZONE (P.P.M.)	ORGANIC NITROGEN (P.P.M.)
		2,000	1,000	500	250	125		
1	2.60	0	0	0	0	0	3.70	0.31
2	2.30	1	0	0	0	0	3.40	0.46
3	0.89	2	0	0	0	0	1.80	0.31
4	0.65	1	0	1	0	0	1.20	0.14
5	0.65	3	1	0	0	0	1.10	0.27
6	0.65	2	0	0	0	0	0.94	0.19
7	0.53	1	*—	2	—	0	1.40	0.69
8	0.53	0	0	0	0	0	1.10	0.29
9	0.53	3	3	2	0	1	0.94	0.37
10	0.30	0	—	0	—	0	0.94	0.71
11	0.30	0	—	1	—	0	0.82	0.44
12	0.30	4	5	3	1	0	0.70	0.64
13	0.17	—	5	3	3	—	0.39	0.56
14	0.17	5	5	5	5	5	0.34	0.50
15	0.17	5	3	0	1	1	0.22	0.14

* No cultures planted.

Results obtained with the treated cysts are presented in Table 3. This table includes only data obtained under essentially similar conditions (19°C., tap water, and pH range of 7.5 to 8.0).

It is apparent from Table 3 that while some positive cultures were obtained with almost all the 5-minute residuals, a high degree of cyst destruction was in evidence with residuals as low as 0.17 P.P.M. For example, with the exception of experiment 14, the numbers of cultures developed from inocula of 500 cysts indi-

⁴ This figure is undoubtedly higher than the number of cysts necessary to establish a positive culture in any given instance, since it is reasonable to suppose that a single, mature cyst would be capable of establishing growth. However, when a series of cultures is inoculated with small numbers of untreated cysts determined by a dilution counting method, certain factors probably operate against the recovery of positive results in all cultures. Among these are sampling errors and the immaturity of some of the cysts. The planting of an average of 10 cysts per culture tube appeared to offset these factors.

cate that in no experiment, regardless of residual, was there an average of 10 viable cysts planted per culture tube. With this same exception, the greatest amount of survival that could be estimated, by comparing the growth in the 250-cyst group with the growth of controls as shown in Table 2, was provided by experiment 13. Only 3 positive cultures were obtained, suggesting that an average of probably not more than 4 viable cysts had been included among the 250 treated cysts planted into each of the 5 cultures. Consequently, even in this experiment a survival of less than 2 per cent of the cysts is indicated.

No explanation is offered for the results obtained in experiment number 14. However, it would have been possible to obtain all positive cultures with a survival of as few as 10 per cent of the cysts. The planting of additional tubes with even fewer cysts would have provided more information in this regard.

It is of interest to note that in experiments 1 to 11, in which the 5-minute residuals ranged from 2.6 to 0.30 P.P.M., there appeared to be no consistent increase in cyst destruction with an increase in ozone residual. Three experiments (numbers 1, 8, and 10) in which no positive cultures were obtained, provided residuals of 2.6, 0.53, and 0.30 P.P.M., respectively. Where positive cultures did occur, they appeared to be scattered somewhat at random throughout the groups containing the greater numbers of cysts, without much correlation with the ozone residual. By comparing the growth resulting from the different cyst inocula in the latter experiments with the control growth (Table 2), the cyst destruction can be estimated at 99 per cent or greater. The greater number of positives obtained in experiment 12 in which the residual was also 0.30 P.P.M., might appear to be an exception. However, the growth in the 500-, 250-, and 125-cyst groups indicates that even in this experiment, cyst destruction was of the magnitude of 98 to 99 per cent.

This high degree of destruction probably explains the failure to get a measurable increase in cysticidal effect with increases in ozone residuals above 0.30 P.P.M. Furthermore, the failure to get completely negative results consistently with the higher ozone residuals, when lower residuals frequently resulted in destruction of 99 per cent or more, suggests that in the former instance there may have been a few cysts more resistant to ozone than the great majority, or that a few cysts may have been protected mechanically by the debris that accompanied the cyst lots.

The organic nitrogen determinations for each experiment are also presented in Table 3. There did not appear to be a consistent correlation between the nitrogen concentration and cyst destruction, as has been demonstrated in tests with other cysticidal agents. This might be explained by the comparatively narrow range of the nitrogen concentrations or by the possibility that with such a high degree of cyst destruction, variations attributable to organic nitrogen would not be apparent. Generally, the 5-minute residual showed a more rapid depletion of the applied ozone in the presence of higher concentrations of organic nitrogen. However, the data are inconsistent in this regard.

The next series of experiments was designed to provide information with respect to the importance of the exposure period. Experiments were set up for

the comparison of cyst destruction 3, 5, and 10 minutes after contact with the ozone in each experiment. Similarly, comparisons were made of 1, 3, and 5 minutes and of $\frac{1}{2}$, 1, and 3 minutes. Residual ozone determinations were made at the various time intervals.

The untreated control cysts were planted in numbers of 20, 10, and 5 cysts per culture tube. The results, shown in Table 4, corroborate the earlier assumption that an average of 10 untreated cysts almost always would produce a positive culture.

Previous results (experiment 14, Table 3) had indicated that the planting of fewer than 125 treated cysts per culture tube would be necessary in order to evaluate the percentage of survival when the latter was 10 per cent or above. In anticipation of possibly greater survival with shorter exposure periods, and in an attempt to find a better method of evaluating cyst survival quantitatively, the logarithmic dilution method of planting treated cysts, previously described as type III, was employed.⁵

TABLE 4

Composite data on the growth of untreated cysts used as controls for experiments on exposure time, temperature, pH, and water source

AVERAGE NUMBER OF CYSTS	NUMBER OF TUBES PER TEST	NUMBER OF TESTS.	TOTAL NUMBER OF TUBES	TOTAL NUMBER POSITIVE	AVERAGE NUMBER POSITIVE CULTURES PER 5 PLANTED
20	5	9	45	45	5.0
10	5	11	55	53	4.8
5	5	10	50	42	4.2

* A single control series served for several experiments when the latter were performed on the same day with the same lot of cysts.

The results of the experiments with varied exposure periods are presented in Table 5. Because ozone concentrations tend to decrease rather rapidly and irregularly within a few minutes, comparisons of effects of varied exposure periods are not made in terms of the ozone residuals. The table is designed for the comparison of results obtained in each experiment at the various time intervals after

⁵ It was originally felt that the growth patterns of the 15 cultures of treated cysts planted in this manner could be compared directly with the growth patterns provided for most probable numbers of bacteria in "Standard Methods for the Examination of Water and Sewage," and, with certain modifications, a calculation made of the most probable numbers of viable cysts present per unit of treated water. However, in view of the small amount of cyst survival, the dilutions of inoculum employed resulted in a lower proportion of positive cultures than that considered as providing the most accurate basis for estimating most probable numbers.

It was felt that, with the data obtained, the use of these tables for estimating cyst survival provided no advantages over the direct comparison of the growth of the treated cysts with that of the untreated controls. However, the addition, to the series of 15 cultures, of a group of tubes planted with larger quantities of inoculum might have provided data more suitable for use with m.p.n. tables.

the application of ozone. The conditions of the experiments in terms of organic nitrogen, temperature, type of water, and pH are included in the table.

The results of experiments 1 to 5 suggest increased cyst destruction at 10 minutes as compared to that at 5 minutes and especially to that at 3 minutes. However, on the basis of a comparison of these results with those obtained with various numbers of untreated cysts, the percentage destruction for the 3-minute exposure was more than 99 per cent; for the 5-minute exposure 96 to more than 99 per cent; and for the 10-minute exposure 97 to more than 99 per cent. These figures indicate that the cyst destruction after 3 minutes was too great to permit a good evaluation of the time factor.

TABLE 5

Growth of cysts of E. histolytica after exposure to ozone in water for various time intervals

EXPERIMENT NUMBER	APPLIED OZONE P.P.M.	NUMBER POSITIVES PER 5 TUBES PLANTED															ORGANIC NITRO- GEN P.P.M.	TYPE WATER	pH	TEMP- PERA- TURE
		½ minute			1 minute			3 minutes			5 minutes			10 minutes						°C.
		1,000*	100	10	1,000	100	10	1,000	100	10	1,000	100	10	1,000	100	10				
1	0.84							1	0	0	0	0	0	0	0	0	0.83	Tap	8.0	19
2	0.53							3	0	0	4	0	0	1	1	0	1.25	Tap	7.9	19
3	0.50							2	0	0	2	0	0	1	0	0	0.50	Tap	8.0	19
4	0.41							4	0	0	4	3	0	3	1	0	0.87	Tap	—	19
5	0.30							4	0	0	1	0	0	0	0	0	0.83	Tap	8.0	19
6	0.88				1	0	0	1	0	0	0	0	0				0.48	D.W.†	7.7	27
7	0.72				2	0	0	0	1	0	1	0	0				0.48	Tap	—	27
8	0.60				1	0	0	1	0	0	0	0	0				0.60	D.W.	7.6	27
9	0.50				3	0	0	2	0	0	3	0	0				0.60	Tap	—	27
10	0.96	3	0	0	2	0	0	0	0	0							1.16	Tap	8.0	19
11	0.72	0	0	0	2	0	0	0	0	0							0.29	Tap	7.6	10
12	0.72	1	0	0	0	0	0	0	0	0							0.29	Tap	7.6	27
13	0.65	5	3	0	3	0	0	1	0	0							0.72	Tap	7.7	19
14	0.65	3	2	0	2	0	0	0	0	0							0.72	D.W.	7.6	19
15	0.55	5	0	0	2	0	0	0	0	0							0.29	Tap	7.3	27
16	0.55	3	1	0	0	0	0	1	0	0							0.29	Tap	7.3	10
17	0.48	2	0	0	0	0	0	1	0	0							0.31	Tap	7.3	19
18	0.48	2	0	1	2	0	0	0	0	0							0.31	D.W	7.5	19

* Numbers of cysts planted per culture tube

† The distilled water was made alkaline by the addition of phosphate buffers.

Four experiments (numbers 6 to 9) were conducted for the comparison of survival after 1, 3, and 5 minutes. In general, results were similar to those in the experiments just discussed. In 3 of the 4 tests fewer positive cultures were observed at 5 minutes than at 1 minute although the differences were not great. Apparently, cyst destruction was relatively high, even after an exposure of only 1 minute.

In experiments 10 to 18, cysts were exposed for ½ minute,⁶ 1 minute, and 3 minutes. There were fewer positive cultures produced from cysts exposed for 3

⁶ Because of the time required for pouring samples, the "½ minute" samples actually contained cysts exposed for from 15 to 40 seconds.

minutes than for $\frac{1}{2}$ minute. However, the notable feature of these results was the high degree of cysticidal action evident even after the shortest exposure period. In only 3 of the experiments were positive cultures obtained when an average of 100 cysts per tube was planted, and in only 1 experiment (number 18) was there growth in a culture planted with 10 cysts. With the exception of the somewhat erratic results of experiment 18, the cyst destruction in all experiments with $\frac{1}{2}$ minute exposure periods was more than 95 per cent.

These experiments with varied exposure time indicate that the cysticidal action of ozone is very rapid. The failure of prolonged exposures to increase markedly this cysticidal action is most likely a reflection of the great amount of cyst destruction occurring within the first minute of exposure.

Aside from such factors as ozone concentration, exposure period, and possibly extraneous material as measured by organic nitrogen, there are other variables

TABLE 6

Growth of cysts of E. histolytica after exposure to ozone in tap water at different temperatures (pH 7.5 to 7.9)

EXPERIMENT NUMBER	APPLIED OZONE (P.P.M.)	EXPOSURE PERIOD	NUMBER POSITIVES PER 5 CULTURES PLANTED									
			19°C.					27°C.				
			2,000*	1,000	500	250	125	2,000	1,000	500	250	125
1	1.10	5	3	1	0	0	0	3	3	1	0	0
2	1.10	5	0	0	0	0	0	0	0	1	0	0
3	0.94	5	3	3	2	0	1	0	1	0	0	0
			10°C.					27°C.				
			1,000*	100	10				1,000	100	10	
4	0.72	1	2	0	0				0	0	0	
5	0.55	1	0	0	0				2	0	0	

* Numbers of treated cysts planted per culture tube.

which might be expected to influence the cysticidal action of ozone. Included among these are temperature, pH, and source of water, i.e., whether distilled or tap water.

Referring again to Table 5, in which the values for these variables are given for the different experiments, it is apparent that the slight differences in growth which did occur cannot be attributed consistently to any certain combination of variables. Such a situation suggests either that the variables in question have no marked effect upon the cysticidal action of ozone, or that the ranges employed were not sufficiently wide, in view of the marked cyst destruction, to provide evidence of such effect.

For the evaluation of the effect of temperature, paired experiments were conducted in which the conditions for each pair were identical except for the temperature. Results of these experiments are presented in Table 6. Experiments

1 to 3 were conducted at the time the type II method of planting cysts was employed. Cysts were exposed for 5 minutes to approximately 1 P.P.M. applied ozone at both 19° and 27°C. Such small variations in growth as can be noted do not appear to be directly referable to temperature. In later efforts to accentuate any possible effect of temperature, experiments 4 and 5 were performed using less applied ozone, a greater temperature difference (10°C. and 27°C.), a shorter exposure time (1 minute), and the type III method of planting. Results in these experiments also failed to show any effect attributable to temperature. Cyst destruction apparently was too great, even at 10°C., to provide significant differences between results at the two temperatures. It is concluded that, at least in the range of applied ozone employed, temperature variation in the 10° to 27°C. range is not an important factor in cyst destruction.

In Table 7 are presented results of some parallel experiments designed to compare effects of ozone on cysts in tap water and in distilled water. The latter was buffered with phosphate buffers to the pH of the tap water, and similar

TABLE 7

Growth of cysts of E. histolytica after 1-minute exposures to ozone in tap water and distilled water (pH 7.3 to 7.7)

EXPERIMENT NUMBER	APPLIED OZONE (P.P.M.)	TEMPERA- TURE	NUMBER POSITIVES PER 5 TUBES PLANTED					
			Tap water			Distilled water		
			1,000*	100	10	1,000	100	10
		°C.						
1	0.80	27	2	0	0	1	0	0
2	0.55	27	3	0	0	1	0	0
3	0.65	19	3	0	0	2	0	0
4	0.48	19	0	0	0	2	0	0

* Numbers of treated cysts planted per culture tube.

amounts of buffer were added to the tap water itself. As is indicated in the table, there was no growth evident in any tubes planted with either 100 or with 10 cysts per tube. The differences in numbers of positive cultures in the 1,000-cyst groups after treatment in tap and in distilled water are not considered significant. In these tests, with applied ozone concentrations in the 0.5 to 0.8 P.P.M. range, the type of water (tap or distilled) did not appear to be a critical factor in the cysticidal action of the ozone. This information is of interest for comparison with the results of Kessel *et al.*, who performed their experiments using buffered distilled water only.

A few parallel experiments were performed to compare the effect of pH upon the cysticidal action of ozone. Values of 6.5 were compared with those in the range of 7.5 to 8.0 in both buffered distilled and buffered tap waters. No significant differences in survival at the two pH levels could be observed.

Although the pH range tested was not sufficiently wide to permit general conclusions, it is apparent that hydrogen-ion concentration within this range is

not a factor of importance. This observation is in accord with those reported by Kessel *et al.*, who found no differences in the action of ozone between pH 6 and pH 9, although at pH 5 the action appeared to be accelerated.

In the experiments reported here, it was found that 97 to more than 99 per cent of *E. histolytica* cysts were destroyed after exposure to applied ozone concentrations of 0.5 P.P.M. and higher for contact periods of 1 minute or longer. Kessel *et al.* obtained apparently complete cyst destruction with a 4-minute residual of 0.3 P.P.M. Actual comparisons of the quantitative elements in the two studies are, however, difficult in view of the differences in technique employed. In contrast to the procedure described in this report, Kessel *et al.* apparently bubbled ozone into the cyst-containing water continuously during the test. Consequently, there are no evaluations of applied ozone concentration suitable for comparison with the foregoing tests in which the cysts were added after application of the ozone. Likewise, ozone residuals after a given period of time cannot be compared directly in view of this difference in ozone application. Further, Kessel *et al.* measured ozone by orthotolidine and expressed the residual values "on the customary chlorine basis," whereas we used the acid starch-iodide method. Consequently, it is not certain whether identical ozone determinations obtained by the two methods are actually expressive of the same concentration of ozone.

In spite of these differences, however, the results obtained in the two studies are in general agreement in that ozone is highly cysticidal in low concentrations and within short contact times.

In the evaluation of a cysticidal agent (3, 4), it has been a common procedure (a) to determine the minimal number of untreated cysts necessary for establishing consistent growth, and (b) to plant only large numbers of treated cysts per culture tube. All negative cultures, then, would signify a cyst destruction in excess of say, 99.9 per cent, the exact figure depending upon the values (a) and (b). However, in instances in which a few or all positive cultures are obtained, this procedure cannot provide information on the amount of destruction other than that it is less than this figure. In the present studies emphasis has been placed upon the development of a method of planting treated cysts in a series of decreasing numbers whereby an estimation of the percentage of cyst destruction is possible when positive cultures occur. The ability to estimate cyst destruction when it is less than complete should permit more complete evaluation of a given cysticidal agent and provide a sounder basis for comparison with other agents.

SUMMARY AND CONCLUSIONS

Cysts of the NRS strain of *E. histolytica* were exposed to various concentrations of ozone in aqueous solution for varying time intervals and under varying conditions of temperature, organic nitrogen, and pH. The criterion of survival of cysts so exposed was their ability to establish growth in culture compared with that of untreated controls.

In 8 preliminary experiments with varying 5-minute ozone residuals, the treated cysts were planted in numbers of 3,000 to 4,000 cysts per tube. Com-

pletely negative results were obtained with 5-minute residuals of 1.8 P.P.M. and above. With lower residuals some positive cultures were obtained. However, the method of planting cysts did not permit estimation of the amount of cyst survival in these instances.

In further experiments with 5-minute ozone residuals, both control and treated cysts were planted in series of decreasing numbers. Results with the control cysts indicated that the presence of an average of 10 cysts per tube almost invariably would result in a positive culture. In addition, growth expectancies were obtained when averages of 8 and 4 untreated control cysts were present per culture tube. These data were used as a basis for estimating cyst destruction when treated cysts were planted in numbers varying arithmetically from 2,000 to 125 cysts per culture tube. The results of 15 experiments indicated a cyst destruction of 98 to more than 99 per cent with 5-minute ozone residuals of 0.3 P.P.M. and above, resulting from ozone applications of 0.7 P.P.M. and above.

In experiments designed to evaluate the importance of contact time, cyst samples were deoxygenated $\frac{1}{2}$, 1, 3, 5, and 10 minutes after application of ozone ranging from 0.3 to 0.8 P.P.M. For the evaluation of the results of these experiments, another method of planting cysts in decreasing numbers was employed, in which the numbers varied logarithmically. The results of 18 experiments indicated that the prolongation of the exposure period beyond 1 minute did not effect a measurable, consistent increase in cyst destruction. It was estimated that 96 to more than 99 per cent of the cysts were destroyed after 1 minute in all experiments, the greater variation in percentage destruction occurring between experiments rather than between the exposure periods of a single experiment.

The following variations in experimental conditions were evaluated with regard to their possible influence on the cysticidal action of ozone: Temperature, 10° to 27°C.; pH in the 6.5 to 8.0 range; distilled water compared to tap water; and organic nitrogen in the range of 0.14 to 1.25 P.P.M. Such variations provided neither consistent nor marked differences in the results obtained, probably because of the high degree of cyst destruction under all conditions employed.

In general, it is concluded that ozone in aqueous solution is highly cysticidal for *E. histolytica* and that the cysticidal action does not appear to be influenced to any great extent by pH, temperature, or organic nitrogen. It is concluded, further, that the evaluation of cysticidal agents by cultural means should employ a method of planting treated cysts in decreasing numbers whereby cyst destruction can be estimated in instances in which it is not complete.

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AMOEBICIDAL ACTIVITY OF BISMUTHOXY *p*-N-GLYCOLYL- ARSANILATE AND 7-iodo-4-(1-methyl-4-diethylamino- butylamino)quinoline diphosphate

E. W. DENNIS,¹ D. A. BERBERIAN AND SOPHIE S. HANSEN

The variety of drugs most commonly used for the treatment of intestinal amoebiasis includes carbarsone, chiniofon, diiodo-oxyquinoline, and emetine bismuth iodide. Emetine hydrochloride is the only drug which has been widely used in the treatment of hepatic amoebiasis, until the recent reports on the efficacy of chloroquine diphosphate (1, 2). Because of the marked tendency of intestinal amoebiasis to relapse after one or more courses of treatment, many laboratories have continued the search for more effective and less toxic amoebicidal agents. It is the purpose of this paper to present the results of *in vitro* and *in vivo* screening tests on certain interesting compounds, with special reference to the behavior of Win 1011² which is bismuthoxy *p*-N-glycolylarsanilate (11) and Win 246 (SN-7620-5) which is the 7-iodo analog of chloroquine diphosphate (Fig. 1).

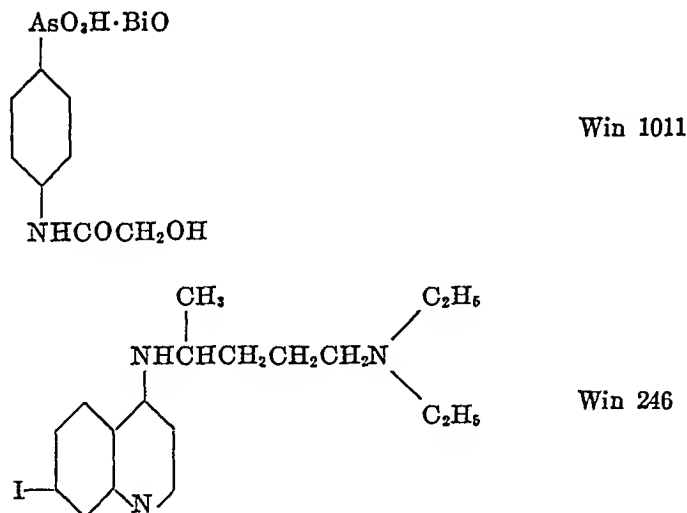


FIG. 1

SCREENING METHODS

In vitro screening procedure: For screening purposes we have been using a strain of *Endamoeba histolytica* obtained from the Division of Laboratories of the New York State Department of Health, Albany. This strain has been maintained on Locke-egg-serum (L.E.S.) medium in association with a mixed bacterial flora. For the *in vitro* screening procedure we have preferred Hansen's (4) egg-in-

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² "Milibis" is the registered trade mark of Winthrop-Stearns, Inc., for bismuthoxy *p*-N-glycolylarsanilate (Win. 1011, Wia).

fusion broth containing a range of dilutions of the drug. Rice starch was always added to both types of media. The inoculum consisted of pooled sediment from a sufficient number of 48 hour L.E.S. cultures to yield a standard suspension so adjusted that one drop contained 400 to 600 amoebae. For test purposes 0.5 cc. of inoculum was added to 4.5 cc. of medicated egg infusion broth. Observations for amoebacidal activity were made after 30 and 48 hours of incubation at 37°C.

In vivo screening procedure: Following the demonstration in October, 1947, that essentially all of the adult hamsters (*Cricetus auratus*) in our colony are more or less heavily infected with a species of *Endamoeba*, it was established that these amoebae are susceptible to adequate doses of known amoebacides and a procedure for *in vivo* screening was evolved.

Test species: The first report of amoebae in hamsters was by Starkoff in 1942 who described the amoebae found in the cecum and colon in two of twelve hamsters examined as *Endamoeba criceti* sp. n. (5). Neal (6) and Fulton and Joyner (7) have reported the presence of amoebae in 50 to 70 per cent of hamsters examined. None of the above authors mentioned the age or size of the animals examined. In a survey made in our laboratory it was found that 17 (55 per cent) of thirty-one hamsters weighing 30 to 35 grams were infected; 55 (96 per cent) of hamsters weighing 35 to 75 grams were infected; and all of 100 hamsters weighing more than 75 grams were infected. For screening purposes hamsters weighing between 90 and 110 grams are selected and used on the assumption that essentially all are infected; nonmedicated controls are examined at frequent intervals to check on the infection rate in the colony but controls have not been included in each screening test. The motile trophozoites of *E. criceti* are most readily demonstrated in mucus scraped from the wall of the cecum; they are commonly present in the colon and occur less frequently in the lower ileum. They are very active, resembling *E. histolytica* in the explosive formation of clear blade-like pseudopodia. The cytoplasm contains food vacuoles enclosing either the remains of associated trichomonad flagellates, which appear to constitute the principal food, or bacteria. The nucleus is prominent, moderately rich in chromatin, and is characterized by a diffuse excentric karyosome. Cysts are rarely seen but have been found in small numbers in iron-hematoxylin stained slides of fecal pellets. The mature cyst has eight nuclei. We have not succeeded in cultivating this amoeba *in vitro*. We have not found any morphological basis for distinguishing the amoebae which we have observed in the hamster from *Endamoeba muris* of the mouse and rat.

Medication procedure: Drugs to be tested for amoebacidal activity are suspended or dissolved in 10 per cent gelatine which has been hydrolyzed at 10 lbs. for 15 minutes in the autoclave. The total daily dose is contained in 1.0 cc. Hamsters are medicated orally by means of a syringe and metal catheter, twice daily for five days. For preliminary screening, three hamsters are treated on each of three or more drug levels based on preliminary toxicity tests; five or ten hamsters are used in each group for evaluation tests.

Evaluation: Medicated hamsters are sacrificed on the day following the last

dose. If at least three slides, each prepared from a different area of the cecum, are found to be free of amoebae the animal is considered as having been cleared.

RESULTS

The results of typical *in vitro* screening tests are presented in Table 1 in a form which illustrates the range of activity encountered in replicate tests. The compounds for which data are given are seen to fall into two distinct groups with respect to range of activity against *Endamoeba histolytica*. The halogenated hydroxyquinolines, chiniofon and diiodo-oxyquinoline, are approximately equal in activity *in vitro*, with average maximum effective dilutions of 1:1000 to 1:1500 under the experimental conditions described. The 7-halogen-4-amino substi-

TABLE 1
RANGE OF AMOEBACIDAL ACTIVITIES IN VITRO
48 HRS., 37°C., IN HANSEN'S EGG INFUSION MEDIUM

SUBSTANCE TESTED	DILUTION OF DRUG									
	1:1000	1:3000	1:5000	1:10,000	1:20,000	1:30,000	1:40,000	1:50,000	1:60,000	1:70,000
CHINIOFON	—									
DIIDO-OXYQUINOLINE	—									
CHLOROQUINE	—	—	—	—						
WIN 246			—	—						
BEMURAL						—	—	—	—	—
WIN 1011						—				
CARBARSONE						—				
EMETINE HCl					—	—	—	—	—	—

tuted quinolines of the chloroquine type are somewhat more active, with average values of 1:3000 to 1:3500. In a distinctly higher zone of activity, which is delineated by the range of variation encountered with emetine hydrochloride, are the two arsenicals, carbarson and Win 1011, and the sulfonamide 3'-5'-dibromosulfanililide (also known as bemural).

Tables 2 and 3 illustrate the effectiveness of the two known therapeutically active amoebicides, carbarson and chiniofon, against the *Endamoeba* sp. of the hamster. Diiodo-oxyquinoline was also active, at somewhat higher dose levels than was chiniofon. Emetine bismuth iodide was ineffective when given orally, even at a drug level of 50 mg./kg. which proved to be lethal within the five day course of treatment. Also, 3'-5'-dibromosulfanililide (bemural) was ineffective at the high level of 1000 mg./kg.

Of particular interest was the relative effectiveness of *p*-N-glycolylarsanilate (Win 1011), and the 7-iodo analog of chloroquine, in clearing the amoebae from the intestinal tract of hamsters. Only a slightly higher dose level of Win 1011 was required for maximum efficiency than was required of carbarsone which is much more toxic. In view of the similarity of behavior *in vitro* of Win 246 and of therapeutically active chloroquine (1, 2), the greater *in vivo* activity of the

TABLE 2
Activity of amoebacidal arsenicals in hamsters

CARBARSONE			WIN 1011		
Daily Dose ¹	Hamsters		Daily Dose	Hamsters	
mg./kg.	Neg. ²	Per cent	mg./kg.	Neg.	Per cent
30	1/5	20	40	0/5	0
40	2/5	40	50	3/10	30
50	3/5	60	75	4/5	80
75	10/10	100	100	10/10	100
0*	0/10	0	0*	0/10	0

* = Controls.

¹ = Administered in 2 doses on 5 consecutive days. Cecum examined for amoebae on the day (6th) after the last dose.

² = Number of hamsters cleared/number treated.

TABLE 3
Activity of amoebacidal quinoline derivatives in hamsters

DAILY DOSE ¹	HAMSTERS NEG. FOR AMOEBAE ²					
	Chloroquine		Win 246		Chiniofon	
	No.	Per cent	No.	Per cent	No.	Per cent
mg./kg.						
150	0/6	0	6/15	40	0/3	0
200	0/6	0	12/16	75	1/8	13
300	0/6	0	6/6	100	7/13	54
400	Lethal		Lethal		5/5	100

¹ = Administered in 2 doses on 5 consecutive days. Cecum examined for amoebae on the day (6th) after the last dose.

² = Number of hamsters cleared/number treated.

7-iodo analog is of considerable interest even though in hamsters a toxic dose of the latter was required to obtain clearance of all animals in the test group.

DISCUSSION

The iodine analog of chloroquine diphosphate, namely, 7-iodo-4-(1-methyl-4-diethylaminobutylamino)quinoline diphosphate (Win 246) was synthesized by Dr. A. R. Surrey (8) and was screened as an antimalarial under the code number SN-7620-5 (9). Win 246 was slightly less effective as an antimalarial than was chloroquine and was not studied extensively until recently. Hoppe and Seppelin

(10) have compared the acute toxicity for mice of Win 246 and chloroquine when administered orally and intravenously in aqueous solution. The oral LD_{50} of Win 246 is 810 ± 50 mg./kg., as compared with the value of 620 ± 80 mg./kg. found for chloroquine diphosphate. The intravenous LD_{50} of Win 246 is 42 ± 2 mg./kg. and the LD_{50} of chloroquine diphosphate is 44 ± 3 mg./kg. According to Wiselogle (9) Win 246 (SN-7620) was found to be six times more toxic than quinine as compared with a quinine index of 5 (Q5) for chloroquine (SN-7618). In our laboratories Hoppe and Seppelin found that eighteen daily doses of 25 mg./kg., administered over a period of twenty-one days, were tolerated by rats without ill effects or deaths. Drug levels of 50 mg./kg. and 100 mg./kg. were tolerated without arrest of growth but deaths occurred (3/9 and 3/10, respectively) at those levels; the survivors were autopsied at the end of the test but no pathological changes attributable to the drug were found. Cumulative toxicity was definitely apparent at a dose level of 200 mg./kg. We have observed degenerative changes in the livers of mice medicated at high dose levels for three weeks. Studies on the absorption, tissue concentration, and excretion of Win 246 have not been completed, but it can be inferred from the lower oral toxicity and the greater amoebicidal effectiveness in the hamster that Win 246 is absorbed less readily from the intestinal tract than is chloroquine. The production of pathological changes in the liver following repeated high doses is presumptive evidence that Win 246 accumulates in the liver as does chloroquine. The circumstantial evidence indicates that Win 246 may be expected to be as active as chloroquine in the treatment of hepatic amoebiasis and may be more effective in the treatment of intestinal amoebiasis. Conan (2) has reported clearance of *E. histolytica* from the stools of 50 per cent of patients treated with chloroquine.

The antiamoebic effect of bismuthoxy *p*-N-glycolylarsanilate was first briefly reported in 1943 by Hauer (11) who called the preparation "WIA". Hauer found that "WIA" was essentially free of toxic properties, was highly effective in clearing stools of *Endamoeba histolytica* cysts in subacute amoebiasis and carriers, but was ineffective in hepatic amoebiasis and in certain cases with very deep ulceration; he reported that "WIA" had a distinct antidiarrheal and sedative effect on intestinal peristalsis in amoebic enteritis. This compound has been synthesized in the Chemistry Division of our laboratories and subjected to extensive investigation as Win 1011.

Win 1011 is a bismuth salt of *p*-glycolylaminophenylarsonic acid, and contains 15.7 per cent arsenic and 37 per cent bismuth. The structure of Win 1011 is shown in Fig. 1. It is a pure white powder, insoluble in water, and almost tasteless. It has not been possible to establish an acute oral lethal dose of Win 1011. Rats have tolerated single oral doses of 10 gm./kg. without any apparent toxic effects. In a twenty-one day subacute toxicity test, Hoppe *et al.* (10) found that rats tolerated daily doses of 5 gm./kg./day without discernable toxicity, and 7.5 gm./kg./day was tolerated with only slight depression of growth rate and without mortality. The drug is well tolerated by humans at a therapeutic dose level of 1.5 gm./day for seven to twelve days when given in divided doses three times a day after meals (11, 12). McChesney (13) has carried out absorption and excretion studies, in rats and human volunteers, which indicate that only 2 per

cent of the therapeutic dose is absorbed from the intestinal tract during ten days of medication. In rats only traces of residual arsenic could be found in the livers, and other tissues were completely free of drug twenty-four hours after the last dose. No pathological changes attributable to the drug were found.

All of the evidence accumulated in laboratory studies indicates that Win 1011 is approximately as active and considerably less toxic than other arsenical amoebicides currently available. In addition to the clinical trial reported by Hauer (11), seventy-two patients have been successfully treated for subacute amoebiasis with a complete absence of side effects (12); of thirty-one patients who received Win 1011 as the only amoebicidal drug, all were promptly cleared of *Endamoeba histolytica* and there were only three recurrences with an average follow-up period of 287 days and an average of ten examinations per patient. In this series one recurrence of *E. histolytica* occurred in the fifth week, one occurred in the fourteenth week, and one occurred in the twentieth week after treatment. The desirable properties of high *in vitro* amoebicidal activity, demonstrated *in vivo* amoebicidal activity, low absorption from the gastrointestinal tract, sufficiently low toxicity to permit administration of relatively large doses for seven to ten days, and a moderate sedative effect on intestinal peristalsis are attributes of Win 1011 which appear to make it an almost ideal drug for the treatment of intestinal amoebiasis. An extensive clinical trial and evaluation of Win 1011 is indicated.

In conclusion, a brief comment on the usefulness of the hamster for the screening of amoebicides is in order. If a heavily infested colony of hamsters is available our procedure offers a convenient presumptive *in vivo* amoebicidal test by means of which inactive or excessively toxic compounds can be eliminated as being *relatively* unpromising. On the other hand, the fact that a toxic dose may be required to clear the hamster of amoebae need not exclude further consideration of a compound such as Win 246 which is fairly well absorbed and of which derivatives with a lower solubility rate can be prepared. It must always be remembered that any laboratory screening test is merely a tool with marked limitations as to the extent to which the results can be used for predicting clinical usefulness. Recommendations for clinical trial of a compound must be based on careful consideration of all available evidence derived from as many kinds of screening tests as possible, adequate toxicity data, and its histotropic behavior in relation to absorption, excretion, and the pathogenesis of the condition to be treated.

SUMMARY AND CONCLUSIONS

1. Bismuthoxy *p*-N-glycolylarsanilate (Win 1011; "WIA") is amoebicidal *in vitro* at a dilution of 1:30,000 to 1:35,000 when tested against *Endamoeba histolytica* in Hansen's egg infusion medium.

2. 7-Iodo-4-(1-methyl-4-diethylaminobutylamino)quinoline diphosphate (Win 246; SN-7620-5) is amoebicidal *in vitro* at a dilution range of 1:3,000 to 1:7,500; it is slightly more active than chloroquine diphosphate (Win 244; SN-7618-5). Both compounds are more active than chiniofon or diiodo-oxyquinoline under similar conditions.

3. Examination of the cecum showed that all hamsters (*C. auratus*) of our colony weighing more than 75 grams were naturally infected with *Entamoeba criceti*, and it was demonstrated that a five day course of oral medication with carbarsone, chiniofon or diiodo-oxyquinoline resulted in elimination of this infestation. It was concluded that the natural infection of the hamster with *E. criceti* is suitable for testing the amoebicidal activity of compounds which might be expected to reach the large intestine in sufficient concentration to be effective in the treatment of intestinal amoebiasis.

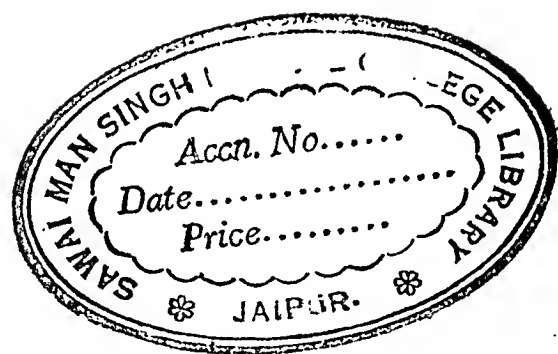
4. Win 246, the iodine analog of chloroquine, is effective in freeing hamsters of their intestinal amoebae when administered at toxic levels; chloroquine is ineffective in hamsters at dose levels which are lethal in five days.

5. Win 1011 is highly effective in clearing hamsters of *E. criceti*. The activity of this drug on a weight basis is greater than that of chiniofon or diiodo-oxyquinoline and is slightly less than the activity of carbarsone. Win 1011 did not produce any evidence of toxicity when administered to hamsters for five days at ten times the dose level required to clear all test animals; carbarsone caused deaths when the dose level was three times greater than that required to clear all test animals.

6. Win 1011 appeared to be completely nontoxic when given orally to humans in therapeutic doses of 0.5 gm three times daily for ten days. In view of the low toxicity and preliminary reports of therapeutic efficacy in the treatment of intestinal amoebiasis it is recommended that Win 1011 be given a more extensive clinical trial and careful evaluation.

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PERCUSSION OF THE SPLEEN¹

BERNARD STOLL²

The views expressed in this paper are derived from studies made during twenty months service in New Guinea with an Army Station Hospital. Well over one thousand patients infected with malaria were personally examined by the author on admission, and those pertinent to this study were followed throughout hospitalization.

Standard textbooks of physical diagnosis do not encourage one to attempt percussion of the non-palpable, enlarged spleen. Neither Major (1), Anderson (2), nor Sutton (3) express an opinion on this point. Buck (4) states, "Percussion of the spleen is difficult and usually unreliable, unless the organ is markedly enlarged." Walker (5) concludes, "... enlargement of the spleen can be said to exist only if the organ is palpable." Siefert and Mueller (6) do consider splenic percussion of value in confirming palpable enlargement, but do not commit themselves concerning its use in determining non-palpable enlargement. Adams (7) accurately summarizes current opinion when he writes, "Some observers believe it possible to demonstrate enlargement of the spleen by percussion, but the consensus of opinion is that enlargement cannot be assumed unless the organ is palpable or roentgenogram shows an enlarged splenic shadow."

The early observations which stimulated investigation of this aspect of physical diagnosis by the author were not planned. Abnormal left lower thorax dullness, not attributable to intra-thoracic disease, was noted on routine physical examination too frequently to be ignored. The abnormality consisted of dullness at a higher level on the midaxillary line than was expected. This was found as high as the sixth intercostal space, and extended downward to the costal margin. Furthermore, the percussion note was often distinctly duller than the impaired resonance which may normally be present below the ninth rib. During this preliminary phase consistently negative chest x-rays forced consideration of an extra-thoracic cause to account for the dullness. The spleen was palpable in many, but not in all of these cases.

Splenic enlargement was tentatively assumed to be responsible for this finding, and every case of fever or suspected malaria admitted to the hospital was studied to test the correctness of this view.

In a large series of patients with palpable spleens, abnormal dullness was almost constantly present. Only five cases can be recalled in which this failed to occur. The fact that in rare instances a palpable spleen is found without evidence of enlargement on percussion, is not unexpected if one recalls the occasional occurrence of a 'floating' displaced spleen.

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Many acute malaria patients in whom percussion furnished the only indication of splenic enlargement were observed. Among these it was common for the spleen to become palpable in a day or two if treatment had not been instituted. In other words, abnormal dullness on the chest wall preceded the development of a palpable spleen.

It was noted, significantly, that barely palpable spleens were associated with about as high a level of dullness as those which were grossly enlarged and easily felt below the costal margin.

At the moment that a barely perceptible spleen becomes inaccessible to palpation, it will still be considerably enlarged. If one could create a clinical situation in which, from this point on, there would be progressive decrease in the size of the spleen, one would have a fine opportunity to study any associated changes in percussion that might occur. This was the case in a large number of patients, never previously exposed to malaria, who were seen during the early days of the New Guinea campaign. Their spleens were not fibrosed by repeated injury; they responded with rapid splenic enlargement during the acute stage, and with prompt shrinkage during treatment. In the latter group of cases, it was found that when the spleen could no longer be felt, a zone of abnormal dullness invariably remained. When this dullness diminished in extent, it did so by descent of its upper border; and in many, abnormal percussion findings finally disappeared.

The dorsal decubitus position has been found to be the most suitable one for this examination. Definite percussion changes may disappear if the patient lies on his right side, and with the patient standing the zone of dullness is diminished. Respiration should be quiet. Percussion of medium intensity is recommended, since forceful percussion can cause failure. I prefer to examine from above downward.

Splenic dullness normally extends from the 9th to the 11th rib in the mid-axillary line (8). A distinct change from pulmonary resonance to dullness occurring at the 8th rib or above, on the midaxillary line, and extending approximately to the costal margin, is a safe criterion of splenic enlargement.

DISCUSSION

This paper is presented to focus attention on the possibility of percussing an enlarged, non-palpable spleen. The performance of this examination is not to be considered as reserved for only a few gifted and experienced physicians. Using the technique and criteria outlined, the necessary information can be elicited easily.

It is important to emphasize that the upper margin of dullness ascends as the spleen enlarges, and may reach its highest level before the spleen can be felt. This observation does not imply that enlargement takes place asymetrically. The lower border of the spleen rests in a hammock, formed by the splenic flexure of the colon and the phrenicocolic ligament, which restrains its downward movement. Therefore, the upper border tends to rise with any increase in size, and this is reflected in an upward extension of dullness on the chest wall. When the

spleen decreases in size, since the lower border rests on supporting structures, the upper border descends; and this, in turn, is reflected in a descent of the level of dullness on the chest wall. I submit this as an explanation for the percussion changes that occur before the spleen has become palpable.

As far as is known to the author, it has not been elicited previously that the most distinctive percussion change associated with splenic enlargement is an upward extension of dullness on the chest wall, occurring before the spleen can be palpated. It is felt that failure to realize the regularity with which this occurs is largely responsible for splenic percussion not being more generally practiced. This permits a simple technique which avoids an attempt to outline the supposed actual size of the spleen.

The point is often raised that the relations of the spleen to the stomach, colon and kidney are likely to make percussion totally unreliable. This would be reasonable if the spleen were a thin strip of tissue. But the adult spleen is normally 3-4 cm. thick (9), and must increase about three times its normal size before it can be felt (10). Thus, there may be considerable separation of the stomach from the chest wall by an enlarged, non-palpable spleen. According to Siefert and Mueller an air-free organ must be 3-4 cm. in diameter, and not covered by more than 5 cm. of air-containing lung to cause impairment of the percussion note (11). The enlarged, but non-palpable spleen satisfies both of these requirements. It is true that forceful percussion may cause undesired contribution to the percussion note from other tissues. But this can be attributed to an unsuitable technique. With this in mind it has been recommended that forceful percussion be avoided. The colon and kidney, situated at the lower border of the spleen, are not likely to disturb the upper margin of dullness which has been stressed as most important. These anatomical relations cannot be as important as is frequently supposed, since percussion evidence of enlargement was easily elicited with great regularity.

A conclusion of splenic enlargement from percussion findings is feasible only if no intrathoracic cause exists that can produce the same signs.

This method of examination has been found useful in spotting atypical cases of malaria in an endemic area, particularly when initial laboratory studies were negative. When the differential diagnosis of a left upper quadrant tumor is difficult, presence or absence of the characteristic zone of dullness on the chest wall may be helpful in deciding whether or not the particular tumor is a spleen. Percussion can be used to advantage when examination of the spleen by palpation is unsatisfactory. This is the case when it is impossible to make the patient breath diaphragmatically because of habitual costal breathing, or when splenic tenderness exists, or when there is present a spastic acute surgical abdomen. Percussion of the spleen increases the completeness of any physical examination, and may contribute to diagnosis by revealing clinically significant splenomegaly before it can be suspected by palpation.

SUMMARY

1. The observations recorded indicate that percussion provides a practicable means of detecting non-palpable enlargement of the spleen.

2. A simple technique of examination, and percussion criteria of enlargement are recommended.
3. Controversial points are considered.
4. The limitations and value of splenic percussion are briefly discussed.

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A MALARIA SURVEY OF THE SOUTHERN TERRITORY OF LOWER CALIFORNIA^{1, 2}

W. G. DOWNS AND E. BORDAS

The Southern Territory of the peninsula of Lower California is one of the least known regions of North America. Until air transportation made access easy, the region could be visited only by boat or by the most arduous of overland trips; and few except explorers, geologists, zoologists and botanists ever visited the region. Mineral wealth attracted geological exploration, and the interesting and often unique flora and fauna of the Cape region south of La Paz attracted scientific expeditions and specialists. There are numerous scientific reports of limited scope. The most comprehensive general report in English is that of Nelson (1), which contains a good bibliography.

GEOGRAPHY AND CLIMATE

The Southern Territory of the peninsula lies between 22°8' and 28° north latitude and 109°4' and 115°2' west longitude. The 28th parallel of latitude divides the southern territory from the northern. Overland communication from the northern territory is possible, but the only road is in poor condition. The entire peninsula is mountainous with a few narrow coastal plains, the largest of which are the Vizcaino Desert in the north and the Magdalena Plain west and northwest of La Paz.

Communication by passable roads exists between La Paz and the towns to the south, namely Triunfo, Santiago, Miraflores and San José del Cabo, and by road to Todos Santos to the southwest. The roads to the towns to the north are nearly impassable, *i.e.* to Loreto, Mulegé and Santa Rosalia, and this trip is usually made by boat.

The climate of the entire southern territory is hot and dry, and most of the region is desert or semidesert. The towns are located where natural springs occur or where water is near the surface. They are actually small oases, often very beautiful, with fine growths of *Washingtonia*, coconut and date palms, plantations of sugar cane, and other crops.

Average annual rainfall in millimeters, during the years 1941-48, for the five stations of La Paz, Mulegé, San José del Cabo, Santiago and Todos Santos, was 198.2 mm., with a minimum of 4.3 mm. for Mulegé in 1947 and a maximum of 586.3 mm. for Santiago in 1943. There is an average of 11 days per year with rain, with minimum of 6 and maximum of 23. The first 6 months of the year are almost without rain, and most of the rain comes in the month of September

¹ The studies and observations on which this paper is based were conducted under the auspices of the International Health Division of The Rockefeller Foundation with the cooperation of the Ministry of Health of Mexico and the government of the Southern Territory of Lower California.

² Read at the meeting of the United States-Mexico Border Public Health Association, April 1949.

(average of 106.2 mm.) in heavy downpours of short duration, sometimes accompanied by storms of hurricane intensity.

The average annual temperature for the five stations is 22.8° C., ranging from an average of 17.1° C. in January to 28.4° in August. Temperatures occasionally go as low as 0.0° C. in January or February and as high as 42° C. in summer.

POPULATION OF RESOURCES

It is estimated that the entire peninsula once was populated by about 25,000 Indians. They have since almost disappeared. The present population, numbering 51,471 (for the southern territory) in 1940, is almost entirely of Spanish descent. Settlements and missions were established late in the seventeenth century. Several of the missions have since been abandoned. Although isolated, the people are progressive, boasting good schools, neat clean towns, often with piped water supply and, in several instances, local health units. The only hospital is at La Paz, although another is under construction at San José del Cabo.

Mining was earlier one of the most important industries of the territory. The famous El Boleo copper mine at Santa Rosalia is still operating, but the silver mines at Triunfo are now closed down. Pearl fishing was important at one time, but has practically disappeared as an industry. The most important pursuits of the territory now are agriculture and fishing. Agriculture is limited to zones near La Paz, Santiago, San José del Cabo, Todos Santos and Mulegé, where there is water sufficient to allow irrigation. The land is very productive, yielding good crops of sugar cane, corn, beans and other vegetables. In recent years, much attention has been given to market gardening of early crops to be shipped to the United States. Shark fishing is a small though important industry, the shark livers being sold for preparation of vitamin products. There is a modern fish cannery at Cape San Lucas, given over chiefly to the canning of tuna.

HISTORY OF MALARIA

Apparently malaria is not an endemic disease of long standing in the region. Dr. Ernesto Chanes (2) states that in 1904 a Sr. Ildefonso Green came from Los Mochis, Sinaloa, to San José del Cabo with malaria, and that since that introduction, the disease has been present in the town. Statistics published by the Health Department (3) show an average annual morbidity rate of 1,205 per 100,000 for the years 1943 through 1947, and an average annual mortality rate of 33 per 100,000 for the years 1940 through 1946. Data from San José del Cabo indicate that the peak of transmission there is in the months of July and August. It must be pointed out that none of the malaria diagnosed in the region has been on the basis of examination of blood specimens, and that therefore the available data does not allow of rigid interpretation.

The malarious towns of the region are reported to be Mulegé, San José del Cabo, Santiago and Todos Santos, and some very small towns adjacent to one or another of the above mentioned communities, and considered as pertaining thereto.

SURVEY DATA

The malarious towns of the territory were visited in July 1948, and spleen and parasite rates were established for school children aged 5-15 years. Spleen size was measured by the method of Hackett (4), and parasite determinations were made from thick blood films stained with Geimsa's stain.

All of the infections encountered were *Plasmodium vivax*. We have no evidence that *Plasmodium falciparum* or *Plasmodium malariae* exists in the region, but it would seem probable that both were introduced in the past and failed to become established as endemic diseases.

TABLE 1

Spleen rates and parasite rates in school children of Southern Territory, Lower California

TOWN	POPULATION (1930 CENSUS)	NO. OF CHILDREN EXAMINED	PER CENT WITH ENLARGED SPLEEN	NO. OF SLIDES EXAMINED	PER CENT PARASITE POSITIVE
San José del Cabo.....	2,638	304	17.5	304	8.2
La Playa (San José).....		60	13.0	60	3.3
Santiago.....	569	101	13.0	101	5.9
Todos Santos.....	1,346	232	18.1	232	6.9
Mulegé.....	802	108	36.1	108	13.0
		805	19.3	805	7.8

TABLE 2

Parasite rates grouped according to spleen size

SPLEEN SIZE	NUMBER OF CHILDREN EXAMINED	NUMBER PARASITE POSITIVE	PER CENT PARASITE POSITIVE
0	650	33	5.1
1	102	15	14.7
2	38	8	21.0
3	15	7	46.7
4	0	—	—
5	0	—	—
	805	63	7.8

ENTOMOLOGICAL INVESTIGATIONS

Anopheles pseudopunctipennis is the only anopheline reported for the southern territory of Lower California and is the only species we encountered. Dr. Luis Vargas examined adults and eggs of specimens we obtained, and he states that they represent *A. pseudopunctipennis* var. *typicus*. In addition to this anopheline, *Culex quinquefasciatus* and *Aedes aegypti* are reported. We also found *Deinocerites epitedeus* Knab at San José del Cabo in crab holes.

Each of the localities visited has special aspects of anopheline ecology, but in general the breeding in each is dependent upon springs, which furnish the

only surface water available in the region and indeed make possible the existence of the towns. In various localities *A. pseudopunctipennis* was encountered in many types of breeding places, but heaviest breeding could always be found in the presence of algal mats. The mats were formed largely of *Spirogyra* and *Hydrodictyon*. Breeding conditions in the four malarious towns are discussed below.

San José del Cabo: There are a series of canals and streams near the town or flowing through the town. All are small, but anophelines were found breeding in several of them. Less than a kilometer from the town, there is a lagoon, 3 to 4 acres in area, shut off from the sea by a sand bar. There are algal mats and emergent vegetation in the lagoon. Anopheline production is heavy.

Santiago: Anophelines are said to occur in irrigation ditches of this town. At the time of our visit, the area was suffering a severe drought, and all the canals were dry. The townspeople say they have sharp outbreaks of malaria following unusually rainy periods.

Todos Santos: There is a dam to the north of, and near, this town, with a pond of about two acres, with emergent vegetation. We found no larvae there, but at times the pond undoubtedly supports heavy anopheline breeding. In a trickle of water at the outlet were numerous larvae. Larvae were also found along the grassy margins of several small canals at the outskirts of the town. In a lagoon of several acres at the shore we failed to find larvae.

Mulegé: There is a dam here, and a pond of possibly 10 acres in area. The entire pond was covered with a mat of green algae, and anopheline larvae were present throughout this algal mat in large numbers (over 100 per dip). Above the pond were several small water holes, only a few square meters in area, where anopheline breeding was occurring. In the stream below the dam, we found larvae in algal mats. We did not examine a lagoon which was open to the sea. In the small water holes above the main dam the water was very calcareous, with deposits of lime at the margins and even on the water vegetation. The water plants were *Chara* sp. and *Najas* sp., and anopheline larvae were abundant.

Searches for house-resting anophelines were conducted in all of the localities visited except Santiago. Houses in San José and Todos Santos, on the outskirts of the town nearest the breeding areas, had densities of 5-10 anophelines per house. In Mulegé, near the dam, there were over 100 anophelines per house.

In addition to the localities listed, we also found *A. pseudopunctipennis* at the Presa San Bartolo, in the mountains between Triunfo and Santiago.

RECOMMENDATIONS FOR MALARIA CONTROL

Barber and Rice's statement (5) that in Egypt they found some evidence that anopheline densities in houses are greater in desert and semidesert regions is of considerable interest in connection with malaria epidemiology and possibilities of control in such regions. Since there are few natural resting places for mosquitoes in the malarious regions of Lower California (there are usually rather open groves of palms and some low shrubbery near the water), it is probable that the anophelines seek houses and man-made shelters. Recommen-

dations were made that a DDT residual spray program be instituted for the region, and this spraying was carried out in September–November 1948, as a joint project of the Oficina de Especialización Sanitaria in Mexico City and the government of the territory. It is too early to assess results of this project as yet. The government of the territory plans to continue this work on an annual basis. It is our feeling that it will result in effective malaria control.

In the course of the survey of breeding places, it appeared that a program of eradication of the anophelines would be possible at relatively moderate expense. Each of the breeding places is separated from the next by 30 to 150 miles, and each place could be handled as a separate unit. Eradication should be possible with the use of larvicidal methods alone, but would be aided by coexisting residual treatment of the houses. There would be very little possibility of reintroduction of anophelines after eradication.

SUMMARY

1. A malaria survey of the southern territory of the peninsula of Lower California reveals endemic malaria (*P. vivax*) in the towns of Mulegé, Santiago, San José del Cabo and Todos Santos. In 805 children examined, the spleen rate was 19.3 and the parasite rate 7.8.

2. *Anopheles pseudopunctipennis* var. *typicus* is the sole anopheline and the vector of malaria in the region.

3. Control of malaria by DDT residual spraying of dwellings is recommended.

4. Eradication of anophelines would be a possibility in the regions visited.

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STATUS OF IMMUNITY FOLLOWING CURE OF RECURRENT VIVAX MALARIA¹

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In 1938 Coggeshall and in 1944 Maier and Coggeshall (1, 2) showed that *Plasmodium knowlesi* in rhesus monkeys could be eliminated by various sulfonamide drugs and that the residual immunity as tested by reinoculation with the homologous parasite was of a mild degree and of relatively short duration. Unfortunately there were no antimalarial compounds which could be relied upon to exert similar action in human malaria until Alving and others (3, 4) demonstrated that pentaquine would eradicate acute and chronic vivax infections. Since these patients were treated and observed in nonendemic area, there was no problem of differentiating relapses from initial infections. Furthermore, all treated subjects were healthy male prisoners who had volunteered to be inoculated by the bites of infected mosquitoes and were still available for observation. Such a combination of circumstances provided an unparalleled opportunity for investigating the status of immunity in human malaria.

MATERIALS AND METHODS

Twenty-one volunteers at Stateville prison, Joliet, Illinois, without previous malaria infections, were inoculated with Southwest Pacific (Chesson) sporozoites of vivax malaria. Some were cured after their initial attack by the administration of 60 mg. pentaquine and 2.0 gm. quinine given daily for fourteen days; others were cured by the same procedure after experiencing several relapses. Following varying intervals after cure, they were reinoculated with sporozoites and trophozoites of both homologous and heterologous strains. The details of each investigation are presented separately.

Homologous Sporozoite Reinoculation. Ten volunteers were subjected to reinfection after cure by the bite of mosquitoes infected with the Chesson strain of *Plasmodium vivax*. They had had from one to seven previous attacks of malaria and from two to fifty-three days of untreated malaria prior to chemotherapeutic cure. The interval between cure and reinoculation varied from three and one-half to twenty months.

Of the ten volunteers, two had experienced only one previous attack; two had had two; three, three; and one each had had four, five, and seven (Table 1).

In those with less than four previous attacks, clinical symptoms, incubation period, and the exuberant multiplication of parasites closely paralleled those observed during their initial primary attack and last relapse. The only change in the status of this group worthy of note was an increase in the pyrogenic

¹ This study was supported jointly by a research grant from the U. S. Public Health Service and the Dept. of Medicine, University of Chicago.

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threshold or minimal density of parasites necessary to produce a fever of 103°F. rectally from an average of 275/cmm for the primary attack, to 400/cmm for the last attack before chemotherapeutic cure, and 500/cmm for the attack following reinoculation. An exception was noted in a volunteer (Moo) who had only three clinical attacks of malaria prior to reinfection but had untreated malaria for a total of fifty-three days, as compared with an average of seven days for the others. An increase in his pyrogenic threshold for 103°F. rectally from 10/cmm with his primary attack to 1890/cmm following reinfection was observed, as well as considerable diminution of symptoms. We might conclude that the only indication of host resistance in those having had less than four previous attacks was the acquisition of a minimal tolerance to parasitemia.

The three volunteers who had experienced four or more malarial attacks displayed considerable immunity when reinoculated and developed only a parasitic

TABLE 1
Homologous sporozoite reinoculation

PATIENT	NO. OF ATTACKS	TOTAL DURATION OF UNTREATED MALARIA IN DAYS	INTERVAL BETWEEN CURE AND REINOCU- LATION IN MONTHS	EVIDENCE OF IMMUNITY
Ros	1	2	20	None
Skr	1	3	16	None
And	2	12	15	None
Ick	7	50	13½	Considerable
Lar	4	21	13	Considerable
Smi	2	5	13	None
Lan	3	7	11	None
Lar	5	23	7½	Considerable
Moo	3	53	7½	Slight
Jan	3	11	3½	None

response to reinfection. Fever over 100°F. rectally was not recorded during a week of parasitemia and symptoms consisted only of mild headaches. Restraint in the multiplication of parasites was observed in all three, and two exhibited a spontaneous disappearance of parasites in eight and nine days, respectively. The parasitemia of the third had begun to decline when therapy was instituted on the eighteenth day. Host resistance here was sufficient not only to demonstrate tolerance to parasitemia but also an ability to destroy parasites.

Interestingly enough, in the group mentioned above, the clinical response was less marked following reinoculation than it was during the last relapse of the original infection. Two of the three developed fever greater than 103°F. rectally during their last relapse, with symptoms of malaise, nausea, myalgia, and headache; whereas, following reinfection all three manifested only a parasitic response. This indicates that there is a residual humoral immunity which is not immediately lost when the continued existence of parasites in the host's body is interrupted.

An attempt was made to correlate the duration of infection prior to cure with

the response to reinfection. In the group subjected to reinoculation with sporozoites of the Chesson strain, the total duration of untreated clinical malaria ranged from two to fifty-three days with an average of eighteen days. In general, those with the greatest number of days of malaria had the greatest number of relapses and showed the most immunity. However, one volunteer was less resistant to reinfection after fifty-three days of malaria and only three attacks than two others who had had less than twenty-three days of malaria but more than four attacks. The interval between chemotherapeutic cure and reinfection ranged from three and one-half to twenty months; however, greater sampling with a better interval distribution would have been advantageous here. From Table 1 it may be concluded that we were unable to demonstrate any significant relation between host resistance and the interval between chemotherapeutic cure and reinfection.

Homologous Trophozoite Reinfection. In order to detect possible differences between sporozoite and trophozoite reinoculations, eight volunteers, previously cured of their mosquito-induced malaria, were each reinoculated by the intravenous injection of whole blood containing 500,000 trophozoites of the Chesson strain of *Plasmodium vivax*. These had, prior to reinoculation, from one to five clinical attacks and from one to twenty-five days of untreated malaria. The interval between chemotherapeutic cure and reinfection ranged from three weeks to nineteen months.

With one exception, no host resistance was noted. In this instance, a change in the pyrogenic threshold of 103°F. rectally from 25/cmm for the initial primary attack to 475/cmm for the last relapse and to 225/cmm following reinfection was observed. The incubation period of the remainder of those reinoculated after cure did not exceed five days and the clinical course was comparable to that of the primary attack. At the end of a week of malaria, when the disease was terminated therapeutically, their parasitemia had not attained its peak and was continuing to show a steady and progressive increase in density. The mean duration of fever of 103°F. rectally per paroxysm in trophozoite-induced Chesson malaria previously had been found to be four and eight-tenths hours; whereas, the mean duration for this group was two and eight-tenths hours. The initial fever following reinfection was of an intermittent character in every case, as contrasted with the usual remittent fever generally observed at the onset of artificially induced Chesson malaria. Thus, in this group of eight reinoculated with trophozoites of the homologous strain, there was little or no evidence of immunity in patients who had experienced one to five attacks of malaria and in whom the interval between cure and reinfection was from 3 weeks to 19 months (Table 2).

Heterologous Strain Reinfection. Three volunteers who had been infected with sporozoite-induced Chesson strain vivax malaria and subsequently had received chemotherapeutic cure were reinfected by the injection of trophozoites of the St. Elizabeth strain of *Plasmodium vivax*.

Two of the men had had three attacks of Chesson strain vivax malaria, and the intervals between therapy and reinfection were thirteen and eighteen months. They were given 500,000 trophozoites of the St. Elizabeth strain intravenously

and promptly developed malaria. Both showed parasitemia within twenty-four hours and febrile response over 100°F. rectally within four days. The pyrogenic threshold for the volunteer who received chemotherapeutic cure thirteen months prior to reinoculation was 15,900/cmm, as compared with 80/cmm for his primary attack of Chesson and 14,000/cmm for his last Chesson relapse. Otherwise, the patient's course resembled that of a nonimmune individual. The institution of therapy on the ninth day of parasitemia prevented our observing any delayed immunological response.

The other case had a pyrogenic threshold of 5,700/cmm for the St. Elizabeth strain, as compared with 120/cmm for his primary attack of Chesson malaria and 5,300/cmm for his attack when given chemotherapeutic cure. Peak parasitemia was attained in nine days. Remission of fever occurred after thirteen days and lasted for six days, after which fever reappeared for three days. At this time his infection was terminated.

TABLE 2
Homologous trophozoite reinoculation

PATIENT	NO. OF ATTACKS	TOTAL DURATION OF UNTREATED MALARIA IN DAYS	INTERVAL BETWEEN CURE AND REINOCULATION IN MONTHS	EVIDENCE OF IMMUNITY
M	1	2	19	None
S	3	8	16	None
Y	3	9	13	None
O	3	11	7½	None
C	4	15	7	None
P	2	7	2	None
H	1	3	2	No response to reinfection*
L	5	25	3 weeks	Considerable

* Second trial resulted in severe infection.

The third volunteer had had eight attacks of Chesson malaria prior to receiving St. Elizabeth trophozoites and previously had been used in immunity studies of the Chesson strain. After seven bouts following sporozoite Chesson malaria, he was given chemotherapeutic cure and later reinoculated by the bite of ten mosquitoes. At this time he exhibited only a parasitic response to reinfection. Again he was given chemotherapy sufficient to eradicate all parasites and after one month was inoculated intravenously with 50 million trophozoites of the St. Elizabeth strain. Within three days he developed parasitemia and on the tenth day had fever. His pyrogenic threshold for 103°F. rectally was 7,040/cmm and his clinical course closely paralleled the others. Peak parasitemia occurred on the tenth day and treatment was started the following day.

DISCUSSION

An excellent and unique opportunity was afforded of conducting an investigation on immunity in volunteers without previous history of malaria who had been inoculated with vivax sporozoites and cured. Following cure, they were

available for long-term observation since they were confined to a prison. The proof of cure seems adequate since the outstanding characteristic of this strain (Southwest Pacific) is its regularity of clinical recurrence. The results quite definitely demonstrated that immunity to a malarial infection exists when the subject is cured and then challenged to a reinfection with the homologous organism. This residual humoral immunity, however, is rapidly lost. There was no evidence of cross immunity with a heterologous strain of the same species of parasite.

Thus, again, it is more than apparent that there is little reason to hope for an effective vaccine for malaria. The above results clearly indicate that in humans, as in experimental animals, resistance to a malaria infection is dependent upon an existing infection, either active or subclinical.

A finding in this study deserving special comment is the greater clinical response upon reinoculation with trophozoites than with sporozoites. This would clearly seem to be a matter of numbers of the infecting organisms rather than an antigenic difference between the two forms of the parasite.

CONCLUSIONS

In this investigation, the following observations appear valid:

1. Following the eradication of acute or chronic vivax malaria (Chesson strain) by chemotherapy there existed temporary immunity to reinfection by the homologous organism.
2. The immune reaction bore a positive relationship to the number of relapses, but not to the duration of infection, prior to cure.
3. Resistance was greater to homologous sporozoite reinfection than to homologous trophozoite reinfection. This was probably due to a greater number of infecting organisms in the latter instance.

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REPORT OF A CASE OF KALA-AZAR RELAPSING AFTER TWO YEARS¹

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Visceral leishmaniasis (Kala-Azar) has been reported with increasing frequency in the United States during the past two years. Search of the literature reveals the appearance of some thirty odd exogenous cases from various sources (1, 2, 3, 4, 5). Doubtless still other cases have been diagnosed and are as yet unpublished. Previous papers have adequately discussed the etiology, epidemiology, pathology, clinical and laboratory findings, and treatment of this disease (1, 2, 6, 7, 8). This report presents a case exhibiting the longest recorded interval between presumptive cure and relapse, two years having passed since "cure" was established and the first appearance of symptoms suggesting recurrence of activity.

REPORT OF CASE

R. G., a 32 year old white male, was admitted to the Veterans Administration Hospital, Washington, D. C., on November 2, 1947, complaining of intermittent fever of about four months duration with recent onset of general malaise and chills accompanying the fever. He had served in the AAF in the China-Burma-India theater from October 1943 to September 1945. In February and March of 1945 he had suffered two febrile episodes diagnosed as dengue, from which he apparently made an uneventful recovery. Early in May 1945 he developed chills, sweats, fever, dull headache, and mild unproductive cough. Hospitalized on May 11th, his examination showed some enlargement of posterior cervical lymph nodes, a few moist rales over the left upper lobe posteriorly, a palpable non-tender liver, a spleen extending two finger-breadths below the costal margin and rapidly developing leucopenia and septic temperature. Laboratory studies were negative for malaria parasites, as were like wise agglutination tests and the chest x-ray, while Leishman-Donovan bodies were discovered in marrow smears obtained by sternal aspiration. He was treated with 3.8 Gms. of Neostam from May 19 to 30 inclusive, resulting in subsidence of fever and splenomegaly, and return of white blood count to normal levels. He was transferred through several service hospitals to the United States, where he was under observation at Moore General Hospital from October 14, 1945 to February 7, 1946. Physical examination, blood count, urinalysis, and blood proteins were normal while in this institution and he was discharged without evidence of disease.

¹ From the Medical Service, Veterans Administration Hospital, Washington, D. C. This paper is published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by us.

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On admission the patient stated that he had been in good health from the time of discharge until June 1947, when he noted on several occasions that his skin seemed to be warmer than usual. Temperature elevations seemed to occur at about weekly intervals until the end of October, when general malaise was experienced, and temperature was recorded at 104 degrees. He then reported to the hospital for diagnosis and treatment.

Physical examination at that time revealed a well developed, well nourished white male, not appearing acutely ill, and weighing 163 pounds (previous weight 175). There was a dusky discoloration of the skin about the face. A non-tender lymph node measuring 0.5 cm. in diameter was palpable in the left supraclavicular fossa and several small nodes were present in the inguino-femoral area. The liver was felt 7 cm. below the costal margin, the edge being smooth and tender. A tender spleen was palpable 8 cm. below the costal margin. Blood pressure was 105 mm. Hg systolic, and 55 mm. Hg diastolic. The remainder of the physical examination was normal. Within the first 48 hours of hospitalization the patient had two chills.

Laboratory Data: On admission the red blood count was 4.24 million, the white blood count 1,900 with 57 neutrophils, 31 lymphocytes, and 2 eosinophiles. Smears were negative for malaria parasites. The Kahn test, stool examination, and complement-fixation tests for schistosomiasis were negative, as were routine blood culture and agglutination studies. Urinalysis revealed only minimal albuminuria. Liver function tests showed 8 per cent retention of bromsulphthalein at 45 minutes, icterus index 8, thymol turbidity 24, cephalin flocculation four plus at 24 hours, and serum bilirubin 0.9. Formol-gel was negative on admission, later becoming positive. Serum proteins on admission were 6.05, with 2.62 gms. albumin and 3.43 gms. of globulin.

The complement-fixation test for leishmaniasis was strongly positive.

Two sternal bone marrow aspirations were done, stained preparations of which failed to show Leishman-Donovan bodies. The material obtained from the first aspiration was not subjected to further study. Material from the second aspiration was injected intraperitoneally into 2 Syrian hamsters and inoculated into culture media. Neither of the hamsters has shown any sign of disease to date. Cultures became positive for *Leishmania donovani* the eleventh day after inoculation in penicillin-free medium and on the 18th day in medium containing penicillin.

Stained preparation of material obtained by splenic puncture revealed the presence of Leishman-Donovan bodies. The same specimen yielded growth of *Leishmania donovani* after 13 days of incubation.

The cultural methods deviated somewhat from those previously employed (16, 17, 18). The standard NNN medium was made into the usual blood agar slants. To some slants 2.5 cc. sterile physiological salt solution was added. To other slants was added 2.5 cc. of salt solution containing 200 units of penicillin per cc. All tubes were inoculated, stoppered with sterile rubber stoppers and incubated at room temperature, which varied between 20° and 24°C.

Since contamination of a specimen is not always unavoidable, it seemed

advisable to use media containing penicillin, owing to the fact that *Leishmania* grow poorly, if at all, in the presence of bacteria (19). Possibly a lower concentration of penicillin than that described should be used. In the bone marrow cultures, there was probably some retardation of growth due to penicillin since the flagellates appeared 7 days earlier in the penicillin-free medium. However, it is thought that isolation of the *Leishmania* would have been more difficult or impossible from the splenic tissue which had been accidentally contaminated, but which yielded growth of the organism in penicillin medium.

This illustrates that failure to demonstrate Leishman-Donovan bodies in stained preparations does not preclude the possibility of obtaining the organisms by cultural methods.

Hospital Course: During the first week, while diagnostic studies were being completed, the patient ran septic-type temperature elevations from 100° to 104°. The second week fever was less prominent, and from then on it subsided by lysis; no further chills occurred. Definite confirmation of the diagnosis having been obtained on the splenic puncture, the patient received a total of 3.9 gms. of Neostibosan from November 13-26 inclusive. No significant toxic effects were noted during the drug administration. The spleen and liver diminished in size, the blood components returned toward normal levels, and the patient gained in weight and well-being progressively during and after therapy. He was followed for several months, receiving an identical "repeat-course" of Neostibosan in January 1948, at which time parasites could not be demonstrated on smear or culture of bone marrow. In February 1948, the liver could not be felt and the spleen was barely palpable. Liver function studies were normal and complement-fixation titers were steadily diminishing. There has been no recurrence of symptoms at the time of this writing, Aug. 1948.

The latent period in kala-azar, *i.e.* time interval from possible infection to development of symptoms, has been variously quoted as ranging from a few weeks to as long as 24 months (9, 10, 11, 12, 13, 14). Relapse, however, has been previously thought to occur within much shorter time limits. Authorities concur that 95 per cent of relapses occur within four months, and 99 per cent within six months. The longest interval before relapse in Most and Laviertes' series (2), was five months. One other report states "—relapse usually occurs within six months but may appear as much as a year later (6)." Relapse is estimated at not over 5 per cent in China and India, though it is difficult to understand how relapse incidence can be accurately determined in endemic areas, where individuals are constantly exposed to re-infection.

This report is presented to re-emphasize the importance of maintaining awareness of the possible appearance of kala-azar in veterans or other personnel returned from endemic areas. Packchanian's (15) study of the distribution of sand flies in the United States, added to the proven possible development of clinical exogenous visceral leishmaniasis after return to the United States, introduces the theoretical potentiality of contracting the disease without leaving this country.

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EXPERIMENTAL TRANSMISSION OF Q FEVER BY AMBLYOMMA CAJENNENSE

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Since Davis and Cox (1) first isolated the causative agent of Q fever from *Dermacentor andersoni*, several species of ticks have been incriminated in the epidemiology of this disease in various parts of the world. Investigations implicating the following species were reviewed by Kohls in 1948 (2): in Australia, *Haemaphysalis humerosa* and *H. bispinosa*, *Ixodes holocyclus*, *Rhipicephalus sanguineus* and *Boophilus microplus*; and in the United States, *Dermacentor occidentalis*, *Amblyomma americanum*, *Haemaphysalis leporis-palustris*, *Ixodes dentatus*, *Ornithodoros moubata* and *O. hermsi*.

Recently Q fever has been demonstrated to occur sporadically in Panama (3, 4). However, there are no data available concerning the possible vectors in this area. We decided, therefore, to attempt experimental transmission by *Amblyomma cajennense*, a tick ideally suited for this purpose in Panama, due to its abundance and wide host range (5). The JD strain of *Rickettsia burneti*, isolated locally (6), was employed as the infective agent and the guinea-pig as the experimental animal.

The identity of the tick-transmitted infection with Q fever was established by testing recovered animals for immunity, transmission of the disease to other guinea-pigs and fertile eggs by inoculation with acute phase blood, and pathological changes in sacrificed animals.

An engorged female *Amblyomma cajennense* was obtained from a dairy cow in the town of Juan Diaz, R.P. on June 10, 1948. It began to deposit eggs on June 16, terminating 3 days later, when it was triturated with saline solution and inoculated intraperitoneally into duplicate normal guinea-pigs, neither of which showed symptoms or subsequent immunity to Q fever.

On July 12, 23 days after the termination of oviposition, the first larval ticks were observed to have hatched. Two groups of 25 each were ground with physiological salt solution for inoculation of paired normal guinea-pigs without production of symptoms or immunity to Q fever.

On August 13, about 100 of the remaining larvae were used to infest a guinea-pig which had been inoculated immediately before with *R. burneti* (JD strain) in yolk sac suspension. The animal showed high fever on August 16 and 17 and died on August 23. Active engorged larvae dropped off the pig on August 20. Typical rickettsiae were observed in stained smears of the gut and its contents in one of these larvae, and inoculation of paired normal guinea-pigs with a saline suspension of 6 triturated larvae readily reproduced the infection in both, whereas 2 immune pigs similarly inoculated remained unaffected.

Nymphal feeding experiments. On September 2 and 3, the larva-nymph moult was completed. A suspension of 2 macerated nymphs in salt solution proved

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fully infectious to two normal guinea-pigs and innocuous to two immunes. Abundant rickettsiae were observed in the epithelial lining cells of the gut and the feces.

Groups of 4 to 25 nymphs were used at various times to infest four normal guinea-pigs. However, only a few, never more than five, were observed to attach to one pig, selecting exclusively the hairless parts of the body. The sluggish engorged nymphs dropped from the host in 5 to 7 days. All four guinea-pigs developed fever of 3 to 7 days duration within 5 to 10 days of infestation. Blood drawn from guinea-pig No. 2 on the second day of fever yielded typical growth in the yolk sacs of chick embryos, and reproduced the symptoms of Q fever in two normal pigs without affecting two immunes. Guinea-pig No. 3 was sacrificed on the first day of defervescence with observation of gross pathological changes

TABLE 1

Results of nymphal-feeding of infected Amblyomma cajennense on normal guinea-pigs

GUINEA-PIG NO.	1	2	3	4
Date of infestation.....	Sept. 13, '48	Sept. 28, '48	Sept. 28, '48	Dec. 2, '48
No. nymphs engorging....	4	3	3	2
First day of fever.....	Sept. 23	Oct. 5	Oct. 3	Dec. 7
Subsequent immunity.....	Immune	Immune	Sacrificed	Immune

TABLE 2

Results of adult-feeding of infected Amblyomma cajennense on normal guinea-pigs

GUINEA-PIG NO.	5	6	7	8	9
No. adults feeding...	1 F	1F & 1M	1F & 1M	1F & 1M	1F & 1M
Date of attachment..	Oct. 21, '48	F—Nov. 5 M—Nov. 6	F—Nov. 16 M—Nov. 30	F—Nov. 18 M—Nov. 11	M & F—Jan. 6 Jan. 12, '49
First day of fever....	Nov. 4	Nov. 10	Nov. 24	Nov. 24	
Subsequent immunity	Immune	Immune	Immune	Immune	Sacrificed

F indicates female, M male.

characteristic of this strain. The surviving pigs all showed complete immunity when tested 12 to 30 days after the onset of symptoms. Data concerning the nymphal feedings are presented in Table 1.

Adult feeding experiments. Of the 12 engorged nymphs obtained in this experiment, 11 moulted as adults in 18 to 20 days yielding 5 males and six females. Considerable difficulty was experienced in inducing these adults to feed. They often remained for long periods in the feeding-boxes without making any apparent effort to attach to the host. Mating was observed to take place in one instance while male and female were attached in the same spot on the animal. Five normal guinea-pigs were infested at various times with 2 to 3 adults each. Febrile reactions of 2 to 5 days duration were observed in all in from 8 to 14 days after attachment of one or more of the parasites. Blood drawn on the second

day of fever from guinea-pig No. 7 was infective to paired normal pigs and to fertile eggs, but did not produce symptoms in 2 immune pigs. Guinea-pig No. 9 showed typical gross pathological changes when sacrificed on the first day of defervescence. The surviving animals were immune to the homologous strain of *R. burneti* when tested 15 to 20 days after the onset of fever. Data concerning the adult feedings appear in Table 2.

Only one completely engorged female was recovered in this series. This female engorged in 12 days and dropped off the host on November 30. It began to oviposit on December 6, terminating on December 13. One of the partially engorged females was macerated for the preparation of smears of the gut and contents, and to inoculate two normal guinea-pigs, with positive results. The others were either killed accidentally or failing of fertilization, engorged only partially and later died.

The first larvae were observed to hatch on December 29, and hatching continued slowly until January 9. Only a relatively small proportion of the eggs yielded larvae. Three normal guinea-pigs were infested with groups of 15, 20 and 20 respectively of these larvae on January 13, 20 and 22, 1949. Small numbers of engorged larvae, 2, 5 and 6 respectively were recovered in 5 to 7 days after application. None of the host animals developed fever or any other symptom and none showed subsequent immunity when tested with the homologous strain of *R. burneti* 19 to 24 days after infestation. Intraperitoneal inoculation of a suspension of 12 triturated larvae in salt solution failed to produce infection or immunity in two normal guinea-pigs, and smears of the gut of three were completely negative.

Thus, no evidence was obtained of transovarian passage of the rickettsia, as successfully demonstrated for *Dermacentor andersoni* by Parker and Davis (7) and for *Ornithodoros moubata* by Davis (8).

CONCLUSIONS

Amblyomma cajennense experimentally infected with *R. burneti* during the larval stage readily transmit Q fever to normal guinea-pigs by feeding during the nymphal and adult stages. Transovarian passage of the infection was not obtained.

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SCHISTOSOMIASIS

F. GORDON CAWSTON¹

When Doctor J. B. Christopherson introduced in the Sudan, the treatment of schistosomiasis with intravenous doses of tartar emetic, he stressed the importance of watching the gradual degeneration of the escaping ova as an estimate of successful cure, and, under the Streatfeild Research Scholarship from 1919 to 1922, I noted the same signs in ova of *Schistosoma mansoni*, *S. bovis* and *S. spindale*.

Sir Leonard Rogers has emphasized the value of the eosinophil count as a means of determining successful treatment, the percentage rising as soon as antimony injections are administered and settling down to normal a few weeks after all the adult worms have been destroyed, but the complement-fixation test has not proved so reliable, and for many the blood count and examination of escaping ova suffice as laboratory tests.

A mere absence of escaping ova may be due to various causes, and is common from some general disturbance of health, so that no reliance can be placed on it in judging that all the adult worms are dead. It is interesting to note that the molluscan host may likewise fail to emit cercariae in the winter months, even though numerous well developed cercariae are found when the liver is crushed.

Positive evidence of the value of tartar emetic in experimental animals given massive doses has been obtained by recovery of the adult worms in various stages of degeneration, and in parts of the body other than their usual habitats. Until similar evidence is adduced for other methods of treatment, their curative value is largely a matter of surmise.

There are however other antimony compounds containing an equivalent of trivalent antimony which have uniformly produced permanent cures, so far as general symptoms and laboratory signs are available, such for example as Anthiomaline, provided the largest possible doses are administered without causing cough or vomiting, preferably intravenously, and continued for a few days after all ova have ceased to escape in the excreta.

It must be borne in mind that all the drug escapes from the system in about two months after a course of treatment has been completed, and, should ova reappear in the excreta at the end of that time, it is probable that the adult worms were not destroyed. In fact experience shows that the possibility of fresh infection is very rare indeed, a permanent immunity arising from the destruction of the parasites.

There is a distinct tendency for the adult worms to die out of themselves, without any treatment, whilst there is some reason to believe that chronic symptoms are as much due to incomplete treatment of cases where the parasites have been displaced from their usual site, or from a concurrent attack of gonorrhoea which is particularly likely to cause serious disease of the bladder and kidney of schistosomiasis patients.

¹ Durban, Natal. Deceased

There is however a relative frequency of slight inflammation of the appendix with schistosomiasis haematobia and, in this connection, it is well to bear in mind the opinion of many who hold that a chronic inflamed appendix does not always justify removal, for pain in the region of the appendix or gall bladder sometimes disappears during a course of injections with tartar emetic, and the adult worms should always be eradicated before operation.

If the appendix is removed before the adult parasites are dead, there may be diaphragmatic irritation immediately following the appendicectomy and tenderness in various parts of the abdomen for some months or years, as I myself experienced under such conditions, while some popular compounds of antimony have a tendency to foster hemorrhage, and on that account, are better avoided in the attempted cure of this disease.

Those doctors who have mastered the technique of tartar emetic injections and even taught it to trained native assistants, while avoiding the large quantities of distilled water which less experienced persons commonly employ, do not experience the toxic effects of a complete course which is often feared by those who have been persuaded to adopt other lines of treatment, nor do they report such large numbers of cases which fail to be cured permanently.

In spite of all that has been brought to light of the ecology of the molluscan hosts in Southern Africa and the means of effecting a complete cure of cases, the disease remains widespread, principally among those living in the native and Indian communities. Unfortunately, while attempting to discourage the breeding of the molluscan hosts, there has been a wholesale disappearance of dragonflies, birds, fish, lizards and other natural enemies.

Occasional hosts of larval trematodes have been ruthlessly condemned, and a species, such as *asbiomphalaria*, which seldom harbors schistosomes except in tropical districts, is rigorously attacked in districts where schistosomiasis is unknown, and where it serves, like many allied molluscs, as a useful scavenger and corrector of the pollution of vleis by the dung of stock and other animals.

Careful study of the records seems to show that *Bulinus forskali* which A. R. D. Adams successfully infected with *Schistosoma haematobium* in Mauritius, has seldom been examined for schistosomes in Africa in those months of the year when it is likely to harbor infection, but that infected specimens have been found at the Natal coast, the only other common molluscan host being species of *Physopsis*.

Among water-cress one may collect numerous examples of *B. tropicus* and *B. natalensis* but evidence that one or both of these molluscs can be regarded as likely hosts in more than one isolated village of the Transvaal is wanting. Identification of cercariae requires recovery of the adult parasites in experimental animals, and unless this is available dogmatic assertions are better avoided.

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LONGEVITY OF *SCHISTOSOMA MANSONI*: OBSERVATIONS BASED ON A CASE¹

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Although the life-cycle and the epidemiology of the three species of schistosomes of major pathogenic importance to man have been studied exhaustively and much is known about these parasites, there yet remains a paucity of information as to the actual possible longevity of these worms in the human host. Yet it is of considerable importance to know how long the adult worm can survive, can continue to throw off living infective ova, and more importantly, maintain the active human infection after removal from an endemic area and from the possibility of reinfection. If the amount of tissue damage caused by continued active parasites could be differentiated from that due to pathologic processes, which once initiated, go on even after the death of all the parasites, important guides to specific therapy would be had.

Such information as to survival times and possible longevity cannot of course be obtained in endemic areas where the possibility of reinfection is ever present. It is best obtained from the sporadic imported case, observed in an area like the continental United States which has no endemic foci of this disease (8). The following authenticated case of prolonged survival of living parasites (over 25 years) is therefore being presented as extending the known life span of the adult of this particular species of schistosome (*mansoni*).

CASE REPORT

(MSH Adm. #583227). This was the first Mount Sinai Hospital admission of a 32 year old Puerto Rican born woman with a seven month history of recurrent attacks of right upper quadrant pain radiating into the epigastrium, straight through to the back, and occasionally around the right costal margin. The attacks occurred about once a week and usually lasted only 1-2 hours. They were related to the ingestion of fatty foods, but still continued after the patient was placed on a fat-free diet. With the attacks of pain, the patient also experienced nausea but did not vomit. There was no fever, chills, jaundice, pruritis, clay colored stools or dark urine. Gall bladder series done in the out-patient department was positive for stones and the patient was admitted for cholecystectomy.

She also gave a history of the occasional passage of blood admixed with her stools. There was no diarrhea. She had been born in a small town, Fajardo, in Puerto Rico, from whence she came to New York City at the age of six. She lived in New York City and its immediate environs uninterruptedly for the twenty-six years since and had at no time returned to Puerto Rico.

Physical examination revealed a well developed 32 year old woman in no acute distress. Temp. was 99.4, pulse 84, and BP 110/70. There was no icterus. Lungs were clear and the heart normal. There was no adenopathy. Abdomen revealed the liver to be at the costal margin with a sharp, thin edge. Spleen was not felt. There was slight resistance in the right upper quadrant with tenderness to deep palpation. Rectal examination was negative. There were no hemorrhoids.

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Laboratory examination revealed a hemoglobin of 12.3 and wbc 5,000. The differential was normal except for an eosinophilia ranging from 2-9 per cent. Urinalysis was normal and the sedimentation rate 10 mm/hr (Westergren). Blood chemical determinations showed the urea nitrogen 12, total protein 6.5 gm. with a normal A/G ratio, ieterus index 3, bilirubin .3, cephalin flocculation and thymol turbidity both negative, alkaline phosphatase 10 King-Armstrong units, and the prothrombin time normal. Blood Wassermann was negative.

Course: Because of the rectal bleeding, the patient was sigmoidoscoped prior to the cholecystectomy. Five inches from the anus on the left lateral wall, a polyp was observed on a pedicle, and it was removed for biopsy. Surgery was then performed and a gall-bladder filled with multisized calculi was removed. The common duct was explored and a small calculus removed from it. The patient made an uneventful convalescence from the surgical procedure.

The rectal polyp was reported by the pathology department as showing "atypical gland proliferation suggestive of carcinoma. Parasitic ova, *Schistosoma mansoni*, present, with inflammatory reaction." Repeated search of the stool, including concentration methods, was negative for schistosome ova. All liver chemistries were normal. There was no evidence either chemically or clinically of schistosome liver cirrhosis. Because of the pathologic diagnosis of possible malignant changes in the polyp the patient was resigmoidoscoped and the base of the polyp fulgurated. Two weeks later repeat biopsy from the granulating wound site in the rectum showed no evidence of carcinoma, but was again positive for schistosome eggs.

Meanwhile, the patient noted that her menstrual period which should have occurred after two weeks of hospitalization was already three weeks overdue. Pelvic examination at this time revealed the cervix softened and the uterus questionably enlarged. AZ test was positive. Because of the pregnancy, therapy directed at the schistosomiasis was deferred and the patient referred to the medical and proctology clinics for continued observation.

DISCUSSION

This patient, born in Puerto Rico, and with ova with the characteristic lateral spines suffered from *Schistosoma mansoni* infestation. The slides were studied by Dr. Ernest C. Faust (9) who wrote "there are several eggs which bear distinctive evidence of mature viable miracidia within the shells. This definitely indicates that there are living adult worms which have recently produced the eggs. If only dead or calcified eggs had been found the question of worms still living would have been unsettled since the eggs might have been deposited a few months previously and tissue reaction might be responsible for the death of the eggs."

Both the patient and her family were questioned repeatedly and it was verified that she had not left the environs of New York for 26 years. Reinfection could not have occurred in this period. Faust (8) in a discussion of the epidemiology of the disease stated "Evidence is completely lacking that a single endemic focus exists in the United States. . . . A more thorough test of the inability of *S. mansoni* to establish itself in North America has been the relatively large number of infected Puerto Ricans who have moved to the United States in the past 35 years. Thousands of infected people have established themselves on the mainland and have offered ample opportunity for the infection to become established but it has not done so." He then adds the warning "From time to time spurious schistosome infections have been reported from the United States or have been submitted to competent parasitologists for confirmation."

Blum and Lilga (5) reported two cases of imported *S. hematobium* infection

in northern Michigan in 1943, and reviewed a total of 33 cases reported in the United States and Canada up to that time. Of those, only one was indubitably acquired in the United States. That case was a four year old boy reported by Sullivan (14) from Chicago. The child developed hematuria and *Schistosoma hematobium* eggs were found in the urine. The manner in which this infection was acquired was indeed puzzling until it was established that he had had access to an aquarium with tropical fish and among these were snails that closely resembled descriptions of the intermediate host, the Egyptian snail *Bullinus*. Examination of the water from the fish bowl showed fully developed cercariae, the infective stage for man. According to the U. S. Public Health Service, this is

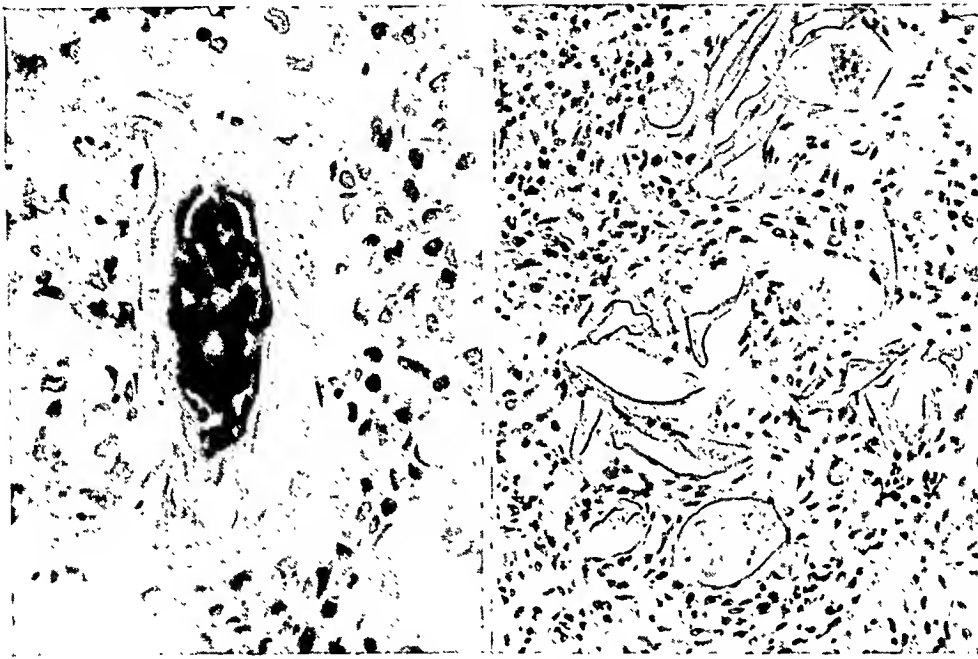


FIG. 1.

FIG. 2.

FIG. 1. OVUM SHOWING LIVING INTERNAL STRUCTURE

FIG. 2. FIELD DEMONSTRATING HEAVINESS OF INFESTATION

the only case described to that date in a person who had never been outside of the United States.

One other reported case falls doubtfully in this category of schistosomiasis acquired in the United States. Peacock and Voegtlin (12) from Seattle reported a 43 year old man with *Schistosoma hematobium* infestation who claimed never to have been outside the United States except for a single trip to Mexico, itself not an endemic area. The origin of this infection remained obscure; possibly it was acquired in a manner similar to that of the four year old.

Occurrences such as these however must be excessively rare and, in view of the evidence adduced by Faust (8) in his very exhaustive epidemiologic survey in relation to this parasite, one must accept that the ova of the patient in this report containing the structures of mature viable miracidia have been recently de-

posited by adult worms alive some 26 years after the patient left the source of infection in the endemic area. Since the flukes cannot reproduce themselves within the body whatever adults are present must be the same ones that developed from the cercariae which penetrated at the time of initial infection.

This is an extension of the known life span of this parasite. The textbooks on the subject, though replete with detailed information on the life cycle of the fluke, treat very sparingly the subject of longevity, especially that of *S. mansoni*. Bercovitz (4), Napier (11), and Stitt and Strong (13) mention nothing on the subject. Ash and Spitz (2) say only that "adult schistosomes may live for years" without qualification as to the species of schistosome or the number of years.



FIG. 3.

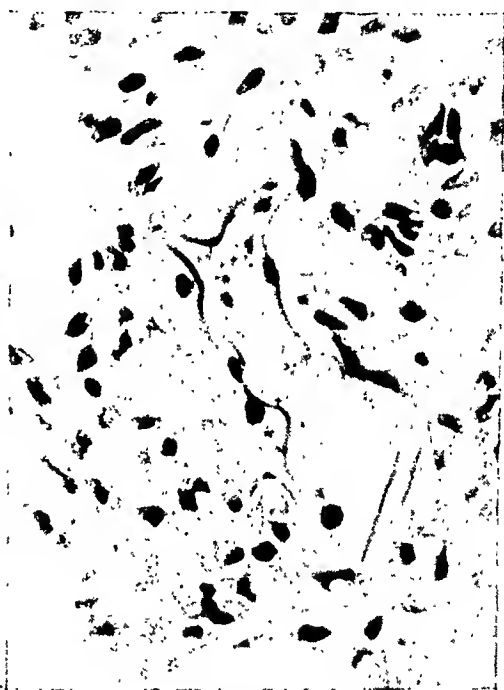


FIG. 4.

FIGS. 3 AND 4. OVA SHOWING WELL-MARKED LATERAL SPINES, DIAGNOSTIC OF *SCHISTOSOMA MANSONI*

There are several references attesting to the long life of *Schistosoma hematobium*. Although Castellani and Chalmers (7) in 1919 stated of *S. hematobium* that "it is probable that the worms may live three years in these vessels, producing during that period large numbers of eggs", Manson-Bahr (10) much more recently (1945) said of the same parasite, "the longevity of this parasite is phenomenal, as it may remain active and produce viable eggs for more than thirty years". He mentioned periods of 20 to 37 years. And Campbell Begg (6) also speaking of *S. hematobium*, stated "Parasites acquired on a single occasion may continue to live and cause symptoms for thirty years or more. Many of course, have a much shorter life. That the Bilharzia fluke can live in the human body for thirty years and more and produce ova is a well-known observation dating from the South African War of 1899-1902. Flukes and ova were found after that period in

soldiers who had never been out of England since their return. I have seen cases in which actual symptoms had been present for 27 years. These symptoms were continuous and the possibility of reinfection was remote."

Comparable evidence on the possible longevity of the other pathogenic schistosomes is much more fragmentary. Faust (9) in a personal communication stated that there are a few records of *S. japonicum* infection over a period of 20-25 years. About *S. mansoni* there is only the case included in Baron's (3) report of his series of patients studied by liver biopsy. In one of these liver puncture revealed *S. mansoni* ova as a hitherto unsuspected finding after which careful search of the stool disclosed viable ova. The report stated that this patient had been in the United States for 30 years, but study of the original hospital chart (MSH Adm. #415909) disclosed the fact that he had come to the United States from Puerto Rico 18 years previously and it was not stated whether he had made any visits back to his homeland in the intervening years. This present report places on record an authenticated case of *S. mansoni* infection with living adults over a period of at least 26 years, an extension of the previous known life span of this parasite.

One other point of interest in this case should be briefly mentioned. The association between schistosome infestation, granulomatous formation, and malignant degeneration has been long known, more especially in relation to *S. hematobium* and bladder carcinoma but also in connection with *S. mansoni* and colonic neoplasm. Afifi (1) in his book, Bilharzial Cancer, says that in 12.5 per cent in one series and in 17.6 per cent in another series of colonic carcinoma (both series compiled in Egypt), the malignant change was found in connection with a polypoid schistosome granuloma. In this case the biopsy was reported only as "suggestive of carcinoma".

SUMMARY

A case of *Schistosoma mansoni* infestation is reported in a 32 year old Puerto Rican woman, who had been out of the endemic area and a resident of New York City for 26 years. Evidence was adduced that this patient had living adult flukes in her body alive at least 26 years an extension of the known life span of this parasite.

Review of the literature reveals that *S. hematobium* has been known to have an equal longevity and that the same has been reported with *S. japonicum* but not as well as can be determined, ever before with *S. mansoni*.

The association of polypoid schistosome granuloma with possible malignant transformation is again noted.

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THE CONTROL OF SCHISTOSOMIASIS JAPONICA

V. STUDIES ON THE PENETRATION OF VARIOUS TYPES OF UNIMPREGNATED UNIFORM CLOTH BY CERCARIAE OF *Schistosoma japonicum*

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INTRODUCTION

It is well known from earlier literature that soldiers acquired schistosomiasis in such areas as Egypt, South Africa, Mesopotamia and China (Laning, 1914; Egan, 1936; and Braune, 1942). However, details of protective measures employed, if any, were lacking. Faust and Meleney (1924) state that the Japanese protected experimentally exposed calves by using waterproof leggings or a double layer of cotton cloth with a mesh of not more than 0.1 mm. As the war in the Pacific turned towards the known endemic centers of schistosomiasis japonica, such as the Philippine Islands, experiments were undertaken by various groups to determine whether or not the available uniform cloth used by the armed forces afforded protection against the penetration of schistosome cercariae. Both the Naval Medical Research Institute and the National Institute of Health became interested in this problem. However, at first only cercariae of *Schistosoma mansoni* were available for experimental purposes. Hill (1945) and Kuntz and Stirewalt (1945), both of the Navy, reported on twelve types of unimpregnated fabrics using *S. mansoni* cercariae. The National Institute of Health, from a series of OSRD reports, appeared to be concerned primarily with the efficacy of impregnated clothing and protective ointments (Churchill, H. M. and Wright, W. H., 1945; Churchill 1945, 1945a, 1945b; Churchill et al. 1945; Nolan, M. D., Roger, M. E., Churchill, H. M., and Jones, M. F., 1945, and others). They employed both *S. mansoni* and *S. japonicum* cercariae.

Preliminary experiments on unimpregnated cloth from Army uniforms were begun early in 1945 by the senior investigator at the Army Medical School using cercariae of *S. japonicum* secured from infected snails shipped from the

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Philippines. This program was interrupted soon after its inception because of an overseas assignment on the Commission of Schistosomiasis of the Army Epidemiological Board. After this group reached Leyte, P. I. in April 1945, it became possible to continue this and related problems.

Recently Ferguson, Graham, Bang and Hairston (1946) reported the results of experiments conducted on Leyte, P. I. in which they studied ointments, and impregnated and unimpregnated uniform cloth. Still later Nolan, Mann and Churchill (1947) reported on a series of experiments with chemically impregnated uniform cloth using cercariae of *S. mansoni* and *S. japonicum*.

The current study was undertaken to determine the degree of protection afforded the wearers of various types of *unimpregnated* uniform cloth, and may be regarded as providing a base line for experiments by other members of the Commission on Schistosomiasis dealing primarily with the penetration of impregnated uniform cloth or infection of animals protected by ointments (Wright, Bauman, and Fry; 1948, 1948a). Using both new and old samples of unimpregnated Army uniform cloth, attempts were made to determine: (1) To what extent cercariae of *S. japonicum* would penetrate regulation Army uniform cloth, and (2) whether or not those cercariae which successfully penetrated the cloth were still capable of infecting a mammalian host. Later several experiments were performed in which the two factors were combined, anesthetized mice being exposed to cercariae in bags made from samples of the same lot of test cloth. By separating the problem into two parts it was hoped to delineate more sharply the role played by each of the two elements—the physical barrier afforded by the cloth and the biological attraction which might be created by the presence of an animal within the cloth.

MATERIALS AND METHODS

Sources of Cercariae: Cercariae of *S. japonicum* were obtained from naturally infected snails, *Oncomelania quadrasi* (= *Schistosomorphora quadrasi*) which were secured from known endemic areas on Leyte, P. I. For the experimental work it was generally agreed that cercariae obtained by natural emergence were preferable to those secured by crushing the snails. In this respect our technique differed from that of Ferguson *et al.* (1946), who used cercariae from crushed snails. As the cercariae normally emerged in quantity late in the afternoon and in the early evening, it was necessary to perform some of the experiments at night. In other cases positive snails were set out in water between 10:00 P.M. and 1:00 A.M., the emergent cercariae being used in the morning.

Apparatus for In Vitro Tests: The tube. The initial test apparatus consisted of a glass tube about 1½ inches in diameter, one end of which was covered with the tightly stretched piece of test cloth, the outer surface of the garment facing the inside of the tube. The test cloth was fastened to the tube with string or rubber bands and sealed with paraffin (Fig. 1). The efficacy of this method of sealing the test cloth over the tube was carefully checked before adopting this technique. The tube was then suspended over an open stender dish by means of a clamp attached to a ring stand. Approximately ten cc. of water containing

50 cercariae were poured from a small beaker into the tube and onto the moistened piece of test cloth. The beaker was rinsed twice with an additional 3 cc. of water and then examined to determine whether any cercariae remained. As the water dripped through the test cloth the tube was lowered until the underside of the cloth was in contact with the surface film. The stender dish was checked at approximately 15 minute intervals up to one hour intervals and the number of viable cercariae that had penetrated the cloth was recorded. Ordinarily, most of the water had passed through the cloth within the first ten minutes.

The windows. It is well known that the cercariae of *S. japonicum* often lie

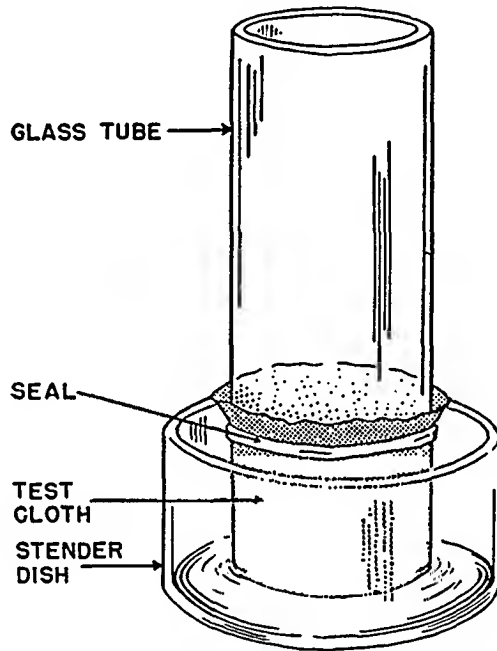


FIG. 1. APPARATUS FOR "TUBE" EXPERIMENTS. (AMDR & GS Photographic Laboratory)

at the surface film. Many workers believe that cercariae penetrate most readily as they lie at the surface film where there may be greater opportunity to secure leverage for penetration as the water evaporates, or where fluctuations of the water surface occur. In order to determine this point another type of *in vitro* experiment was designed. A window was cut in a bamboo tube and the test cloth was sealed over the window with the outer surface of the cloth facing the outside. The entire margin was sealed with paraffin. The bottom of the tube was also covered and sealed with a sheet of cellophane thus making it possible to examine the interior of the tube by transmitted light under a dissecting microscope (Fig. 2). The bamboo tube with the sealed cellophane end down was placed in a beaker containing a known number of cercariae. Sufficient water was present to fill the bamboo tube to the middle of the window when equilibrium was reached by seepage of the water through the piece of cloth. The water

inside the tube was examined at intervals and the number of cercariae that had penetrated the cloth was recorded.

Other in Vitro Experiments: Other modifications of these two fundamental types of *in vitro* experiments were made.

Effect of pressure. The effect of pressure on the cloth covering the glass tube was tested by rubbing the surface of the cloth inside the tube for a period of ten seconds with a glass rod following the addition of cercariae. In this way it was hoped to simulate the stretching and spreading of the interstices that would occur when the knee or elbow was flexed.

The effect of pressure on test cloth was also checked by utilizing glass sputum cups. The test cloth with its outer surface uppermost was placed over the open end of the cup and held firmly in place by means of rubber bands or string. The cercariae were poured into the cloth and the water permitted to drip through

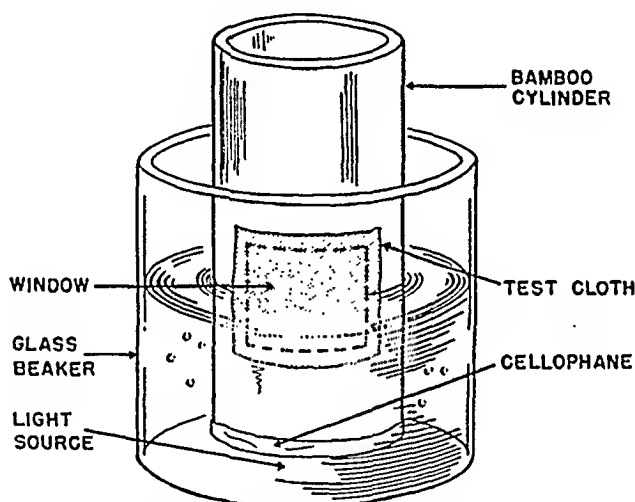


FIG. 2. APPARATUS FOR "WINDOW" EXPERIMENTS. (AMDR & GS Photographic Laboratory)

(Fig. 3). During this process the surface of the cloth was rubbed for ten seconds or more with a glass stirring rod. Subsequently the water that collected in the bottom of the cup was examined for cercariae. The results of all of these experiments on pressure were not included in the summary of the data (see Table I) on the protection afforded by the various types of uniform cloth since they would have introduced another variable, i.e. pressure.

In Vivo Experiments: Direct Exposure. In order to determine whether or not cercariae that had penetrated pieces of the test cloth were still sufficiently viable to infect a mammal, the cercariae were harvested and mice were exposed to them. The cercariae were placed in a 500 cc. beaker and checked for motility. After the number of motile cercariae was recorded a normal mouse was added and allowed to remain in the half inch of water in the beaker for at least 30 minutes. An attempt was then made to detect any cercariae remaining. The mice were sacrificed in four to six weeks and examined for schistosomes by the method described by Nolan, Mann, and Churchill (1947).

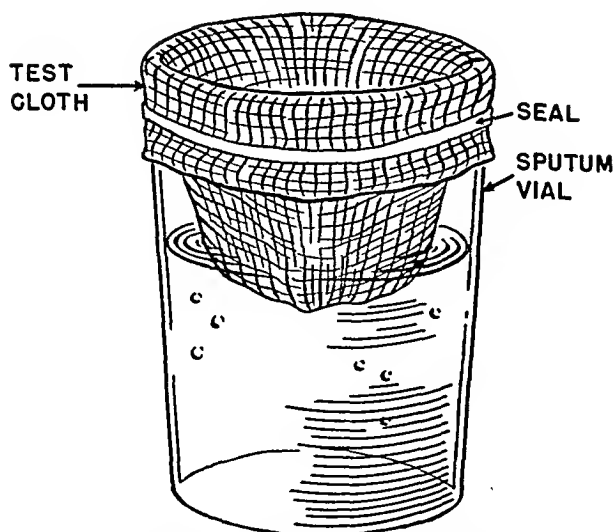


FIG. 3. APPARATUS FOR SPUTUM CUP EXPERIMENTS. (AMDR & GS Photographic Laboratory)

TABLE I

In vitro tests

Relative protection offered by various types of army uniform cloth to the penetration of cercariae of Schistosoma japonicum

TYPE OF FABRIC TESTED	NO. OF EXPTS. RUN	NO. POSITIVE	POSITIVE	NO. OF CERCARIAE USED	CERCARIAE RECOVERED	
					Total	Penetra- tion in all tests
			<i>per cent</i>			<i>per cent</i>
Water resistant sateen: new.	33	0	0	1650	0	0
Water resistant sateen: washed 1 to 4 times.....	60	3	5.0	3000	3	0.1
Fatigues: new, herringbone twill.....	50	18	36.0	2610	32	1.23
Fatigues: old, herringbone twill.....	35	10	28.5	1750	12	0.69
Cotton trousers: new, khaki twill.....	44	20	45.4	2200	26	1.18
Cotton trousers: old, khaki twill.....	30	8	26.6	1500	14	0.93
Woolen O.D. trousers: new..	30	11	36.6	1500	24	1.6
Woolen O.D. trousers: old...	39	22	56.4	1950	80	4.1
Cotton shirt: new, khaki twill MacArthur type....	33	12	36.3	1650	27	1.63
Cotton shirt: old, khaki twill MacArthur type....	47	33	70.1	2250	121	5.38
Woolen O.D. shirts: new....	47	36	76.6	2350	102	4.34
Woolen O.D. shirt: old.....	39	31	79.4	1950	211	10.82
Controls—2 layers of Curity Gauze.....	25	25	100	1250	782	62.5

Controls: Controls for tube and window experiments. In the tube and window experiments controls consisted of two layers of Curity Gauze which were substituted for the test cloth. The number and percentage of cercariae successful in penetrating the gauze was recorded. Additional controls for these experiments consisted of samples from the different lots of cercariae used in the experiments which were set aside at the start of the experiment and were examined for viability when the experimental runs were completed. As the latter were always alive, they were not recorded in the tables.

Controls for direct exposure. Controls consisted of exposing normal mice in the same fashion as described above to *freshly* shed cercariae and examining for schistosomes at autopsy.

Controls for bag experiments. A second type of *in vivo* experiment was used primarily as a control. This consisted of making bags with French seams from the different test cloths and then placing anaesthetized mice in these bags for approximately an hour. These bags were suspended in 500 cc. beakers half full of water containing 100 viable cercariae of *S. japonicum*. The mice were autopsied at the end of four to six weeks and the number of adult schistosomes recorded.

Types of Army Uniform Cloth Tested: The uniforms to be tested were secured largely from the Quartermaster Depot on Leyte, P. I. One lot of old fatigues (herringbone twill) jumper type, and the old cotton shirt (MacArthur type), came from members of the Commission. The latter was about 3½ years old, having been purchased in early 1942. Regardless of the source, the following types of new and old (salvage) Army uniform cloth were tested.

Herringbone twill (from fatigues)

Cotton khaki twill (from shirts)

Cotton khaki twill (from trousers)

Olive drab woolen (from shirts)

Olive drab woolen (from trousers)

Water resistant sateen, new (M-1903-9 oz.)

A minimum of 15 different samples of new and old cloth were tested with each type of apparatus. The percentage of cercariae that successfully penetrated the cloth in the individual tests was then calculated (see Table I).

PRESENTATION AND DISCUSSION OF RESULTS

In Vitro Experiments: Results of cloth penetration experiments. A total of 487 tests employing the types of apparatus depicted in Figures 1 and 2 were run on 12 different types of uniform cloth. In 204 of these tests the cercariae penetrated the cloth barrier. In all a total of 24,360 cercariae of *S. japonicum* were utilized but only 652, or 2.63 per cent, succeeded in penetrating the various pieces of the test cloths, compared with 62.5 percent in the case of the Curity Gauze controls (see Table I). This indicates clearly that even though 41.8 per cent of the tests were positive, a high degree of protection was afforded by unimpregnated Army uniform cloth since comparatively few cercariae actually succeeded in penetrating the cloth. In this connection it should be borne in

mind that greater numbers of cercariae were used than probably would be encountered by an average individual as long as he remained in uniform and exercised other indicated control measures.

The summary of the experiments on each type of cloth appears in Table I. The greatest degree of protection is afforded by the Army's water resistant sateen which proved to be virtually waterproof. After four washings with G. I. soap, each of five minutes duration, a few cercariae, 0.1 per cent of the total used, apparently penetrated the cloth. It was not possible to continue these washings to the point where the cloth broke down to permit the passage of any significant numbers of cercariae. Old herringbone twill (fatigue cloth) and old cotton khaki twill (trousers) also afforded excellent protection, as less than one per cent of the cercariae succeeded in penetrating the cloth. New woolen O.D. trousers gave fair protection as only 1.6 per cent of the cercariae penetrated the interstices of the cloth. However, cloth from old woolen O.D. trousers passed over 4 per cent of the cercariae.

*Statistical significance of the data*⁶. It is always desirable to know the statistical significance of data where possible. The standard error of the difference was computed on some of the data listed in Tables I and II. In such cases a figure of $\frac{\bar{X}}{\sigma}$ that approaches or passes 2.0 is usually assumed to be significant. First

the new and old types of cloth were compared. The results on those approaching significance follow: cotton khaki twill trouser 1.7, woolen O.D. trouser 1.68, cotton khaki twill shirt 3.16, (cf Table II). A similar comparison between old herringbone twill and old woolen O. D. trouser material was 2.5 while the old cotton khaki twill trouser and old woolen O.D. trouser was 2.7.

The significance of these last two figures is of considerable interest since the results obtained indicate that old woolen O.D. trouser material does not give as good protection as either the old herringbone twill or the old cotton khaki trouser material. In this connection it should be pointed out that our findings in relation to the woolen trouser material are not in accord with those of Ferguson et al (1946) or Nolan et al (1947), who claimed a greater protective value for woolen O.D. trouser material than for herringbone twill or cotton khaki trouser twill. However, they present no statistical data to support their figures. It appears possible that the discrepancy in the results may be attributed to the variations in samples used.

Factors influencing results. In all of these experiments involving the use of a cloth barrier, the dripping of water through the interstices of the cloth must have been an important factor in facilitating the passage of cercariae. This simulates the conditions obtained when an individual first wades in water. However, once equilibrium was reached, passage of cercariae must have been due largely to their own activity. In most instances where penetration was observed, the number of cercariae was found to increase after equilibrium had been established.

There are several factors to be considered in attempting to evaluate these and

⁶ Thanks are due to Mr. L. V. Phelps, PH & W, SCAP for running the statistical analysis of these figures.

comparable experiments reported by other workers. Some factors are the tightness of the weave of the cloth, the variation in the thickness of the individual threads used by different mills, the effect of shrinkage, or wear, as well as the number of washings. In addition, any imperfection in the weave or rents in the cloth merit consideration as well as differences in the technique of testing. Furthermore, in discussing the different results obtained by various groups, the species of cercariae which were used must be considered.

Weave of the cloth. Individual differences between mill lots are allowed within Army specifications, and these may account in part for the differences noted in various lots of experimental cloth as reported by Hill (1945), Hunter (1945) and Ferguson, Graham, Bang and Hairston (1946). There was no possible way to insure adequate comparisons of two lots of cloth from different sources. For example, one test lot (Fig. 7) was taken from an old shirt of the MacArthur type. The material and weave were never matched in new regulation shirts of the same style purchased subsequently. Consequently results recorded by other workers cannot be accurately compared.

TABLE II
Per cent of cercariae penetrating different types of cloth

	WATER RESISTANT SATEEN	FATIGUES HERRINGBONE Twill	KHAKI TROUSERS, COT- TON Twill	WOOLEN O.D. TROUSERS	KHAKI SHIRT COTTON Twill	WOOLEN O.D. SHIRT
New.....	—	1.23	1.18	1.6	1.63	4.34
Old.....	0.1	0.74	0.93	4.1	5.38	10.82

Photomicrographs of four different types of test cloth appear in Figures 4 to 7. Marked differences in the interstices (which appear white in the figures) of the different types of cloth may be seen. Figure 4 shows the impervious nature of the new water resistant sateen even when compared with other types of Army uniform cloth. Figure 5 shows old cotton trouser khaki twill. Old worn khaki twill shirting that is well over three years of age appears to be quite porous (Fig. 7) as does the old woolen O.D. trouser material (Fig. 6). These findings are in essential agreement with those secured by Ferguson, Graham, Bang and Hairston (1946) even though different techniques were employed and different cloth samples tested. The principal difference occurred in the samples of woolen O.D. cloth. However, when our samples were tested by these experimentors they too noted cercarial passage although their woolen cloth had been resistant (Ferguson et al, 1946). In view of this apparent discrepancy in results obtained, including those of Nolan et al (1947), it is obvious that the differences obtained must have been due primarily to differences in the woolen cloth samples. Therefore, it appears unwise to generalize upon the degree of protection afforded by woolen O.D. trouser material lest a sense of false security be created.

Stretching and shrinkage. It is well known that many types of cloth stretch considerably when wet. The exact degree of "give" in the various types of cloth tested is not known, but this might well explain why cercariae succeeded

in penetrating some types of cloth which appear to be relatively non-porous when dry. Conversely in other cases there may be a swelling of the fibers when

FIG. 4.

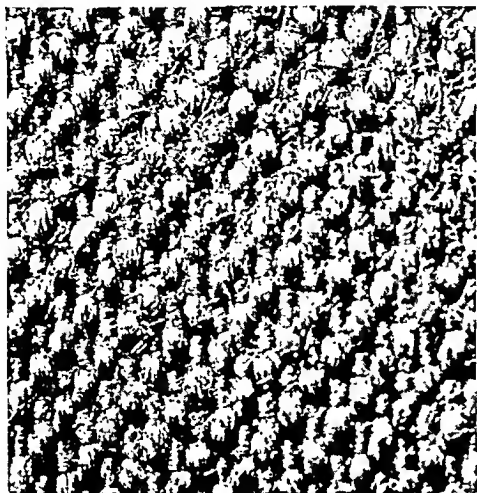


FIG. 5.

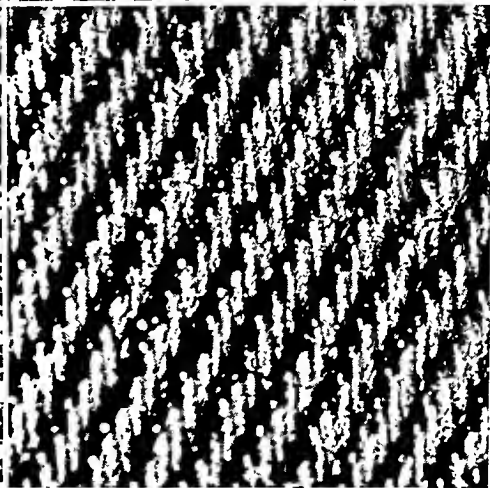
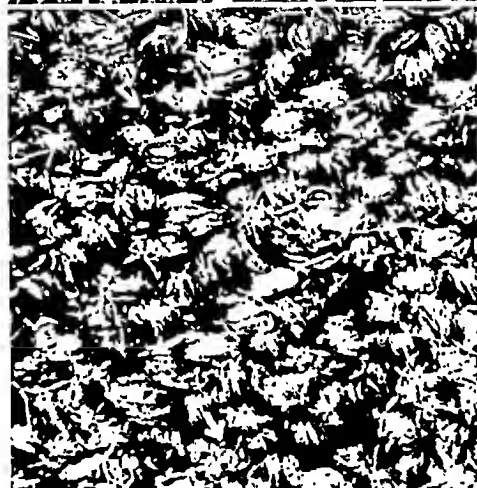
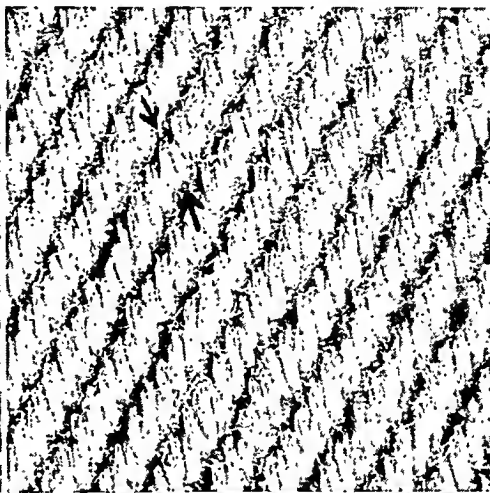


FIG. 6.

FIG. 7.

FIG. 4. WATER RESISTANT SATEEN WASHED FOUR TIMES FOR FIVE MINUTES WITH G.I. SOAP
Note the absence of the white dots which indicate interstices. $\times 8$. (Army Institute of Pathology Negative No. 99469).

FIG. 5. OLD COTTON TROUSER KHAKE TWILL

Note the presence of only a few interstices. (cf. Figs. 4 and 7). $\times 8$ (Army Institute of Pathology Negative No. 99475).

FIG. 6. OLD WOOLEN O.D. TROUSER MATERIAL

Because of the thickness of the cloth and the nature of the weave it is difficult to see the interstices which are present. $\times 8$. (Army Institute of Pathology Negative No. 99466).

FIG. 7. OLD KHAKE COTTON SHIRT MATERIAL

Note abundant interstices (cf. Figs. 4, 5, and 6). $\times 8$. (Army Institute of Pathology Negative No. 99477).

moistened, thus reducing the size of the interstices and so preventing the passage of cercariae in quantity.

Types of washing. It also appears probable that repeated washings, especially under field conditions, would detrimentally affect the wearing quality of the test cloth. In the Philippines, the native washwomen washed by beating the uniforms with paddles on rocks. This treatment was undoubtedly much harder on clothing than an Army laundry. Consequently, aside from all other variables, this factor alone might result in two pieces of cloth, originally from the same mill, furnishing entirely different results when finally tested as samples since one lot might have been subjected to harsher treatment. Furthermore, even if new cloth is aged artificially in the laboratory by repeated washings, the end results achieved by two different sets of investigators might vary because of the difference in the "aging" technique.

In addition to the variables mentioned above, one should remember that protection in the field would be determined in some degree by the extent and duration of immersion and whether or not the uniform was intact.

Species of cercariae. Much of the early experimental work on impregnated and unimpregnated cloth was carried on of necessity with cercariae of *S. mansoni* (*vide supra*). This is the largest of the three cercariae producing schistosomiasis in man. In performing experiments with one species there is always the danger of generalizing on the results obtained. The danger inherent in so doing can be readily inferred from Table III which lists the measurements of the cercariae of each of the important human species.

This table suggests that different results in the ability of cercariae to penetrate cloth might well be expected if cercariae of *S. mansoni* and *S. japonicum* were carefully tested using the same techniques. Assuming activity of cercariae to be the same and other factors also being equal one would expect *S. japonicum* to penetrate cloth in greater numbers than *S. mansoni* since they are smaller and therefore presumably capable of working through smaller interstices.

Experiments to determine the effects of pressure on penetration. A series of experiments was attempted to simulate the type of mechanical pressure on the cloth that would occur when a knee or elbow joint was flexed. Cercariae were poured onto the test cloth which was tied over the open end of sputum cups. The filtrate was examined at intervals following the application of pressure and the number of cercariae recorded (Table IV). Comparative data were obtained only in the case of new herringbone twill (fatigue cloth) and new woolen O.D. shirt material. However, reference to Table IV indicates that in both cases a significantly greater number of cercariae penetrated the cloth that was placed under pressure for ten seconds. These experiments indicate that pressure tends to facilitate the passage of cercariae through the interstices of the cloth.

In Vivo Experiments: The penetration of test animals by cercariae. From the results obtained in the experiments outlined thus far it is evident that under laboratory conditions limited numbers of cercariae succeeded in penetrating the various types of Army uniform cloth that were tested. The next question centered around the ability of the cercariae which successfully penetrated Army uniform cloth to infect man, or some other susceptible animal. White mice were selected as the test animals. Unfortunately, it was impossible to test sep-

arately the cercariae that had penetrated *each type* of Army uniform cloth since in most cases sufficient numbers of cercariae did not succeed in penetrating. However, enough tests were completed overseas and in the United States by one of us (G. W. H., III) to demonstrate that cercariae which penetrated Army uniform cloth were still sufficiently active to infect mice. Table V summarizes the results obtained in these *in vivo* experiments which represent this second phase of the problem. Some of the negatives in Table V undoubtedly would have been positive if the experimental animals had been exposed to greater numbers of parasites.

Bag experiments. Control infection experiments on anaesthetized mice that were placed in bags of test cloth were carried on. The technique employed

TABLE III

Comparison of measurements in micra of cercariae of three species of Schistosomes
(After Craig and Faust 1945)

	BODY LENGTH	BODY WIDTH	TAIL LENGTH	TAIL WIDTH	FURCAL LENGTH
<i>S. mansoni</i>	185-230	75-110	185-300	60-75	90-130
<i>S. japonicum</i>	100-160	40-66	140-160	20-35	50-75
<i>S. hematobium</i>	140-240	57-100	175-250	35-50	60-100

TABLE IV

Showing the effect of pressure on the ability of cercariae to penetrate cloth

CLOTH	NUMBER OF CERCARIAE USED	PRESSURE APPLIED TO TEST CLOTH	NUMBER OF TESTS RUN	NUMBER OF TESTS POSITIVE	TOTAL NUMBER OF VIABLE CERCARIAE RE- COVERED
New herringbone twill...	1180	No	15	3	4
New herringbone twill...	800	Yes	15	13	59
New O.D. woolen shirt..	750	No	15	11	28
New O.D. woolen shirt..	750	Yes	15	13	70

follows. The hair was clipped from the sides of animals anaesthetized with sodium amytal. These were placed inside bags made from the same lots of test cloth used for the *in vitro* experiments and the bags were suspended in beakers of water containing known numbers of cercariae (*vide supra*). The water gradually seeped through the cloth until it reached equilibrium with that on the outside of the bag. Table V clearly indicates that cercariae which penetrated all types of cloth were still sufficiently viable to infect mice. The bag experiments were designed to determine whether the presence of animals *within* the bag during immersion would attract *more* cercariae and so result in a heavier infection of test animals. No significant difference could be detected (cf. Tables V and VI). This suggests that the presence of the animal within the bag did not exert a detectable chemotropic influence upon the cercariae. Further evidence of the lack of a chemotropic response lies in the fact that the viable cercariae of *S. japonicum* placed on the skin of man or mice do not penetrate until

the water starts to evaporate.⁷ This is in agreement with the observations of Faust, Jones and Hoffman (1934) on the penetration of the cercariae of *S. mansoni*. It is believed that the cercariae of *S. japonicum* respond to drying by attempting to penetrate the object with which they are in contact. It is apparent from Table VI that there is not a statistically significant difference (when the number of cercariae used is considered) in the number of adults recovered in those animals exposed in bags of cloth and in those which were exposed to mice following penetration of the cloth (Table V).

TABLE V

Summary of in vivo tests following penetration of cloth by cercariae†*

CLOTH	NO. OF ANIMALS USED	NUMBER OF CERCARIAE USED	NUMBER OF WORMS RECOVERED AT AUTOPSY	AVER. NO. PER ANIMAL
None.....	10	100 in 5; 50 in 5	15, 22, 6, 0, 28, 11, 7, 14, 0, 6	1.9
Curity Gauze.....	5	47, 40, 44, 42, 41	11, 3, 7, 29, 10	12.0
Herringbone twill, new.....	5	23, 5, 4, 9, 10	0‡, 0, 0, 1, 1	0.5
Herringbone twill, old.....	5	2, 3, 7, 11, 9	0‡, 0, 0, 0, 2	0.5
Khaki cotton twill trousers, new.....	3	20, 11, 13	2, 1, 2	1.7
Khaki cotton twill trousers, old.....	2	16, 7	1, 0	0.5
Woolen O.D. trousers, new.....	3	4, 3, 12, 17, 16	0, 0, 1, 4, 1	1.2
Woolen O.D. trousers, old.....	2	50, 42	6, 4	5.0
Khaki cotton twill shirts, new..	2	22, 5	2, 1	1.5
Khaki cotton twill shirts, old..	3	84, 18, 10	20, 0, 4	8.0
Woolen O.D. shirt, new.....	1	32	3	3.0
Woolen O.D. shirt, old.....	2	71, 34	0, 0‡	9.0

* Some of these were run after the return of the senior author to the AMDR&GS, AMC, Washington, D. C.

† In passing it should be pointed out that these data, although limited, are of interest because *exact* counts were made on the numbers of cercariae used.

‡ Died, not examined for worms, nor included in the calculations.

Cloth protects the wearer. It will be seen from Table II that the test Army uniform cloth may be grouped into four classes in accordance with the degree of protection offered. The water resistant sateen is in a class by itself as it proved experimentally to be virtually impervious to cercariae. The next group includes both herringbone twill and the cotton khaki trouser material, while the third group offers considerably less protection and consists of O.D. woolen trouser and cotton khaki twill shirt material. The woolen O.D. shirt material is the

⁷ Based upon personal observation by one of us (G. W. H., III).

least resistant of all types tested to the penetration of cercariae of *S. japonicum*. These figures clearly indicate that a very considerable degree of protection is afforded at least for short periods of time to the wearer of any uniform cloth (Hunter, 1945).

While the differences in the techniques used make further comparisons difficult it is clear that any properly worn uniform will afford considerable protection to the wearer. In this connection the fact that cotton khaki or herringbone twill uniforms are generally worn during the periods of greatest snail activity, i.e. the warm weather, should be kept in mind. In most areas this probably also means the period of greatest production of cercariae. The relative effectiveness of these materials as a mechanical barrier to the passage of cercariae of *S. japonicum*

TABLE VI
Summary of in vivo bag tests

BAG COMPOSED OF	NUMBER OF ANIMALS	NUMBER OF PARASITES RECOVERED AT AUTOPSY	NUMBER OF WORMS PER ANIMAL
Controls—Curity Gauze††	5	16, 1, 18, 2, 14	10.2
Herringbone twill—new	5	0, 2, 1, 1, 5	1.8
Herringbone twill—old	5	0, 0, 2, 0, 1	0.6
Cotton khaki twill trousers—new	5	1, 2, 0, 4, 4	2.2
Cotton khaki twill trousers—old	5	0, 0, 1, 2, 2	1.0
Woolen O.D. trousers—new	5	0, 6, 2, 8, 6	4.4
Woolen O.D. trousers—old	5	3, 10, 2, 8, 7	6.0
Water resistant sateen	5	None	—

* Approximately 100 cercariae were used in each test. Test animals (mice) were anaesthetized before placing in bag.

† Two layers of gauze.

‡ Thirteen mice exposed to 200 cercariae each by Wright, Bauman and Fry in connection with their studies yielded 9.9 worms per animal at autopsy.

means that the person who wades in cercaria-infested water secures considerable protection. This certainly is true provided the bottom of his trousers are tucked into the top of his combat boots thus preventing the passage of large volumes of water directly under the bottom of the trousers to come into direct contact with the skin. Where prolonged exposure to cercariae might occur as in the combat engineers and other such groups (Sullivan and Ferguson 1946) protection would not be so satisfactory.

SUMMARY AND CONCLUSIONS

In view of the experimental evidence presented in these pages the following points are made:

1. A total of 204, or 41.8 per cent, of the 487 tests run on samples of Army

uniform cloth were positive for the passage of small numbers of cercariae of *S. japonicum* through the cloth.

2. The actual number of viable cercariae of *S. japonicum* that succeeded in effecting a penetration of the samples of Army uniform cloth tested in the *in vitro* experiments was low. Only 652, or 2.63 per cent, of the 24,350 cercariae used in these experiments penetrated the cloth compared with 62.5 per cent of the controls.

3. The results of the *in vitro* experiments show that the different types of cloth permitted the passage of cercariae in increasing numbers in the following order: Water resistant sateen—new; water resistant sateen—washed four times; herringbone twill—old fatigue cloth; cotton khaki twill—old trousers; cotton khaki twill—new trousers; herringbone twill—new fatigue cloth; woolen O.D. trousers—new; cotton khaki twill—new shirt; woolen O.D. trousers—old; woolen O.D. shirt—new; cotton khaki twill—old shirt; woolen O.D. shirts—old.

4. Evidence is presented to show that when pressure is applied to pieces of test cloth greater numbers of the tests become positive and greater numbers of cercariae are successful in penetrating the cloth.

5. The recovery of adults of *S. japonicum* from mice exposed to cercariae that had successfully penetrated samples of test cloth suggests that these parasites are still capable of infecting man.

6. Adult schistosomes were recovered from mice that had been exposed to cercariae-infested water protected only by bags of test cloth. The number of adults recovered was essentially the same as those recovered after exposure to cercariae that had penetrated the cloth in the *in vitro* experiments.

7. It is evident from the foregoing experiments that all types of Army uniform cloth afford some degree of protection to the wearer. Herringbone twill (fatigue cloth) and khaki cotton twill would appear to give slightly better protection than new woolen O.D. material. However, *any* uniform trouser material would protect the legs provided it was tucked into the top of combat boots and enough slack was present to form a cuff over the boot top.

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PHOTOMICROGRAPHS OF THE DEVELOPING LARVAE OF *WUCHERERIA BANCROFTI* IN A MOSQUITO HOST OF THE SOUTH PACIFIC AREA¹

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The photomicrographs accompanying this paper were made during experimental work on filariasis supplemental to the preliminary experiments reported by the author in a previous paper (1). An experiment with microfilariae of *W. bancrofti* had been attempted using the fifth and sixth generations of laboratory-reared *A. p. punctulatus* and *A. p. farauti*. Two natives from the Island of San Cristobal with respective counts of fifty-nine and sixty-eight microfilariae per 20 cu. mm. of blood, fed the mosquitoes which were housed in two separate cages. Dissections made each day revealed that the worms picked up by *A. p. punctulatus* did not survive longer than the fourth day. Larvae were found alive and developing normally in *A. p. farauti* at the end of the seventh day. The mortality rate of the mosquitoes was high and by the end of the seventh day the colony had been completely wiped out. It was thought that the cause of this high death rate might have been due to the fact that the later generations of laboratory-reared mosquitoes are weaker physically and are more susceptible to changes in temperature and other conditions which would not materially effect first and second generations reared in the laboratory. Although the dissections showed that the larvae of *W. bancrofti* did not survive for many days in these specimens of *A. p. punctulatus* it was not considered conclusive evidence that this species is not involved in the transmission of filariasis. It was decided to use blooded mosquitoes from native villages for dissection and other experimental work.

EXPERIMENTAL WORK

A collection of blooded mosquitoes was made during the early morning hours from huts in native villages where cases of filariasis were known to exist. The insects were transported to the laboratory where identification of species was made by Capt. John Belkin, SnC, former entomologist of this Unit. Under Captain Belkin's supervision most of the mosquitoes were kept alive and allowed to lay eggs before being dissected. All mosquitoes were examined for filaria larvae and when possible to extract the stomachs an examination was made for oocysts. The results of the dissections are shown in Table 1.

It will be noted from the table that only two of the two hundred fifty-seven mosquitoes examined for oocysts were positive and both of these specimens were *A. p. punctulatus*. It was not demonstrated that the oocysts were of human origin.

¹ The author is indebted to Lt. Robert Reiber, SnC and Lt. John Chattin, SnC for their very capable assistance in carrying out the laboratory technique on Guadalcanal 1944-1945.

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Filaria larvae were encountered in all phases of development including the infective stage. Fully developed larvae were found in the abdomens of seven mosquitoes and were assumed to be larvae of *D. immitis*. Infective stage larvae of *W. bancrofti* and *D. immitis* were found in the same mosquito in several instances. The incidence of *W. bancrofti* and *D. immitis* is shown in Table 2.

For the purpose of obtaining a complete series of photomicrographs of developing larvae, approximately one thousand eggs from the *A. p. farauti* caught in the villages were carried through to the adult stage. Two cages were used to house the mosquitoes and five hundred were placed in each cage. A period of five days elapsed before they received their first blood meal. On the fifth night between the hours of 1930 and 2000 the same two natives used in the previous experiment (1) fed the mosquitoes. On the following morning, and every morning

TABLE 1

Incidence of filaria larvae and oocysts in blooded mosquitoes found in native villages on Guadalcanal

SPECIES	OOCYSTS			FILARIA LARVAE		
	Dissected	Positive	% Infection	Dissected	Positive	% Infection
<i>A. farauti</i>	145	0	0	195	54	27.69
<i>A. punctulatus</i>	112	2	1.78	200	30	15.00
<i>Armigeres brucei</i>	0	0	0	2	0	0
<i>M. uniformis</i>	0	0	0	3	0	0

TABLE 2

Incidence of infection with W. bancrofti and D. immitis found in mosquitoes

SPECIES	<i>W. bancrofti</i>	<i>D. immitis</i>
<i>A. p. punctulatus</i>	30	0
<i>A. p. farauti</i>	47	7

thereafter, dissections were made at the same hour. The larvae developing in the mosquitoes were photographed each day. The mortality rate of the mosquitoes was low and many were alive on the seventeenth day after feeding, at which time all remaining mosquitoes were killed.

All dissections showed that in less than twenty-four hours most of the worms picked up by the mosquitoes had migrated to the thorax and were exsheathed. Several worms found in the stomachs at the end of twenty-four hours were still very active and later dissections seemed to indicate that some of these straggling worms eventually reach the thorax and develop. One mosquito dissected on the tenth day after feeding was found to harbor larvae in different stages of development. Measurements showed them to be .48 mm.; .72 mm. and .86 mm. in length (see Figures 12, 13, 14).

PHOTOMICROGRAPHY

Photomicrographs of living, unstained larvae were found to be superior to photomicrographs of stained specimens. For microfilariae in blood smears Bulard's hematoxylin with cosin counter-stain was excellent.

It was necessary for the author to improvise several attachments for use with the Speed Graphic camera. The lens and lens board were removed and in their place was substituted a piece of corrugated cardboard with a short piece (one inch in length) of adhesive tape spool (Figure 20-B). The spool was covered on the inside with black friction tape to prevent reflection of light from the metal surface of the spool. A light-tight collar for use with the microscope was made from a larger diameter and longer piece of adhesive tape spool and was covered on the outside with adhesive tape (Figure 20-C). A tightly fitting sleeve of tubular cardboard was fitted over the collar so that it could be slid up and down. The ground glass focusing plate on the camera was too coarse for sharp focusing and was replaced by a piece of cellulose acetate with one etched surface. A frame of corrugated cardboard was made the same size as the frame for the glass plate and the sheet of acetate was taped onto the frame with the etched surface up (Figure 20-A). A thin film of vaseline was applied to this surface to increase definition and sharpness of the image. The light source was a 200 watt mazda lamp. All of the photomicrographs were made using a "G" filter between the light source and the mirror of the microscope. The camera was placed in a vertical position on a wooden box into which a hole had been drilled, and fastened securely by the knurled screw of the tripod holder. The box was screwed to a heavy table. The bellows of the camera was lowered to within about two inches of the microscope eyepiece. The sleeve on the light-tight collar was then raised to within a fraction of an inch of the substituted lens board so there was no actual contact between camera and microscope although light leaks were prevented. This also eliminated vibrations which would have caused the image to go out of focus when the focusing screen was removed and replaced by the film pack. As soon as the slide was removed from the film pack, exposure was made by turning the lamp on and off with a snap switch. An exposure of four seconds was found to be satisfactory with Agfa Superpan film and the "G" filter. All photographs were made by contact printing.

To demonstrate more graphically the development of the parasites, the author chose to photograph all stages at the same magnification using a $6\times$ ocular and 4. mm. objective. To determine the exact magnification on the film, photomicrographs were made of a micrometer scale and it was found that with the lens system used a magnification of $240\times$ was obtained. The problem of photographing the later stages of developing larvae, when it became necessary to use several fields for an examination of the entire specimen, was overcome by using the method employed in making ariel photographs. Each field was photographed separately until the entire length of the worm had been covered. An overlap was allowed at each exposure. The negatives were then matched, cut and the sections were fitted together to form what might be termed a "micro-mosaic".

SUMMARY

1. From the experimental work with *A. p. punctulatus* it appears that this species of mosquito is a vector of filariasis in this area.

2. The first generation of mosquitoes reared in the laboratory is probably much more hardy than later generations and is less liable to show a high rate of mortality.

3. It is quite practical to make photomicrographs without all of the attachments generally considered necessary for this type of work. Such equipment is expensive and unavailable in most areas and may be improvised.

4. It is believed that this is the first time that the complete larval development of *W. bancrofti* in a mosquito host has been photographed at a magnification which demonstrated structural detail and that this system of "micro-mapping" might be used more often under similar circumstances.

REFERENCE

- (1) "Observations on the incidence of *Wuchereria bancrofti* larvae in the native population of the Solomon Islands area." Am. J. Trop. Med. Vol. 25, No. 6, pages 493-495.

PLATE 1

- FIG. 1. Infected natives feeding mosquitoes for use in experiment.
- " 2. Stained specimen of *W. bancrofti* showing sheath (blood smear). (550 X).
- " 3. Larva from thorax of mosquito, 18 hours old. Note absence of sheath. (240 X).
Approx. .29 mm long. (240 X).
- " 4. Larva 24 hours old. Approx. .29 mm long. (240 X).
- " 5. Larva 1½ days old. Note shortening, thickening and spiked tail. (240 X).
Approx. .19 mm long.
- " 6. Larva 2½ days old. Approx. .15 mm long. (240 X).
- " 7. Larva 3½ days old. Approx. .18 mm long. (240 X).
- " 8. Larva 4½ " " " .22 " " (240 X).
- " 9. Larva 5½ " " " .32 " " (240 X).
- " 10. Larva 6½ " " Prior to moulting. Approx. .30 mm long. (240 X).
- " 11. Larva 7½ days old. After moulting. Note moulted skin on posterior end. Approx. .30 mm long. (240 X).
- " 12. Larva 8½ days old. Approx. .48 mm long. (240 X).
- " 13. Larva 9½ " " " .72 " " (240 X).
- " 14. Larva 10½ " " " .86 " " (240 X).
- " 15. Larva 11½ " " Note great increase in size. Approx. 1.24 mm long. (240 X).
- " 16. Larva 12½ " " Approx. 1.53 mm long. (240 X).

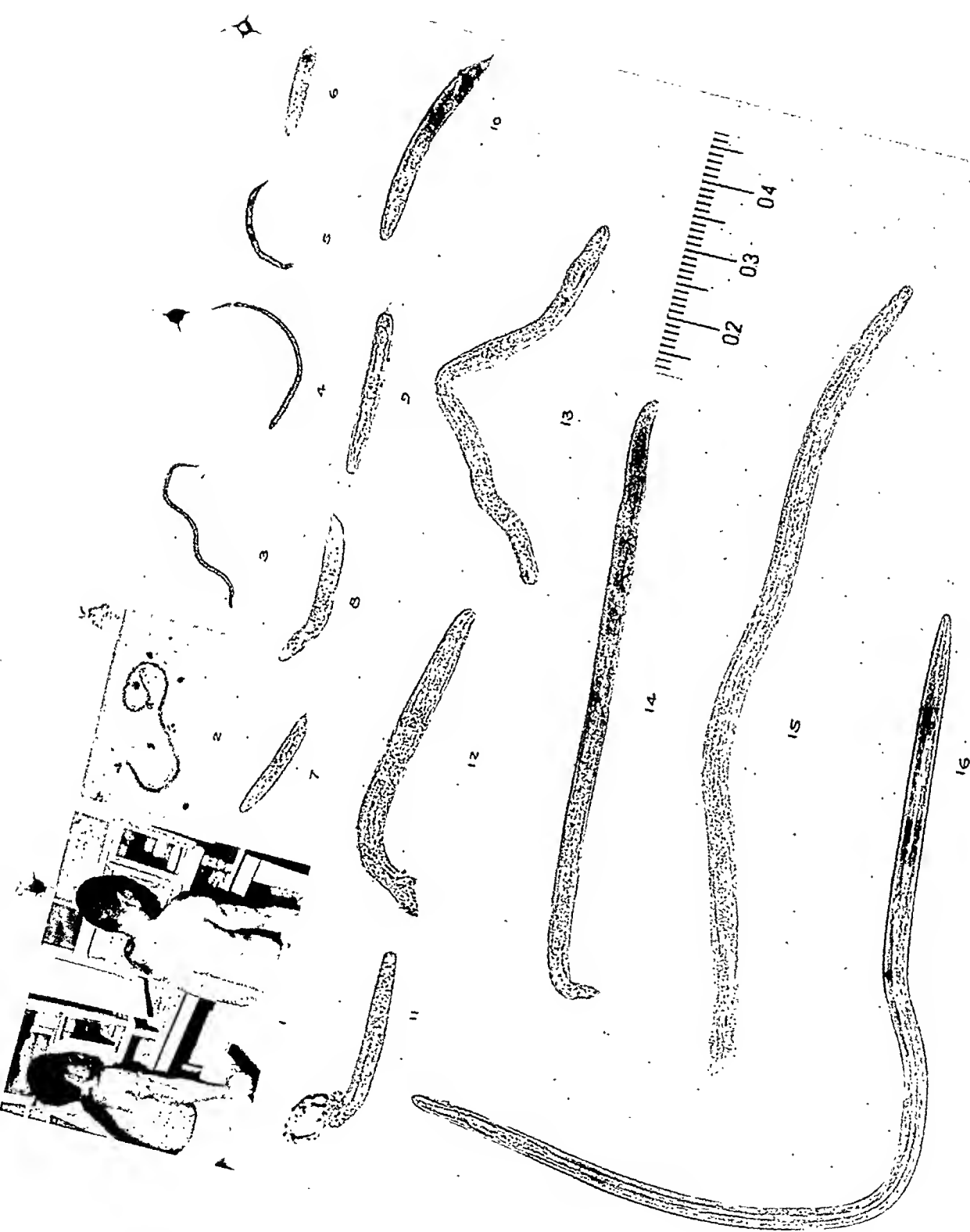


PLATE I

PLATE 2

- FIG. 17. Larva $13\frac{1}{2}$ " " Approx. 1.75 mm long. (240 \times).
" 18. Larva $14\frac{1}{2}$ " " Infective stage. Approx. 1.80 mm long. (240 \times).
" 19. Infective stage larvae in and around labium of mosquito. (55 \times).
" 20. Complete set up for photomicrography.

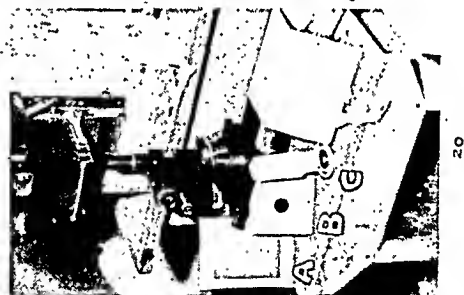
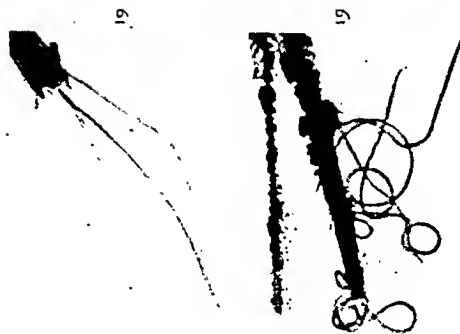
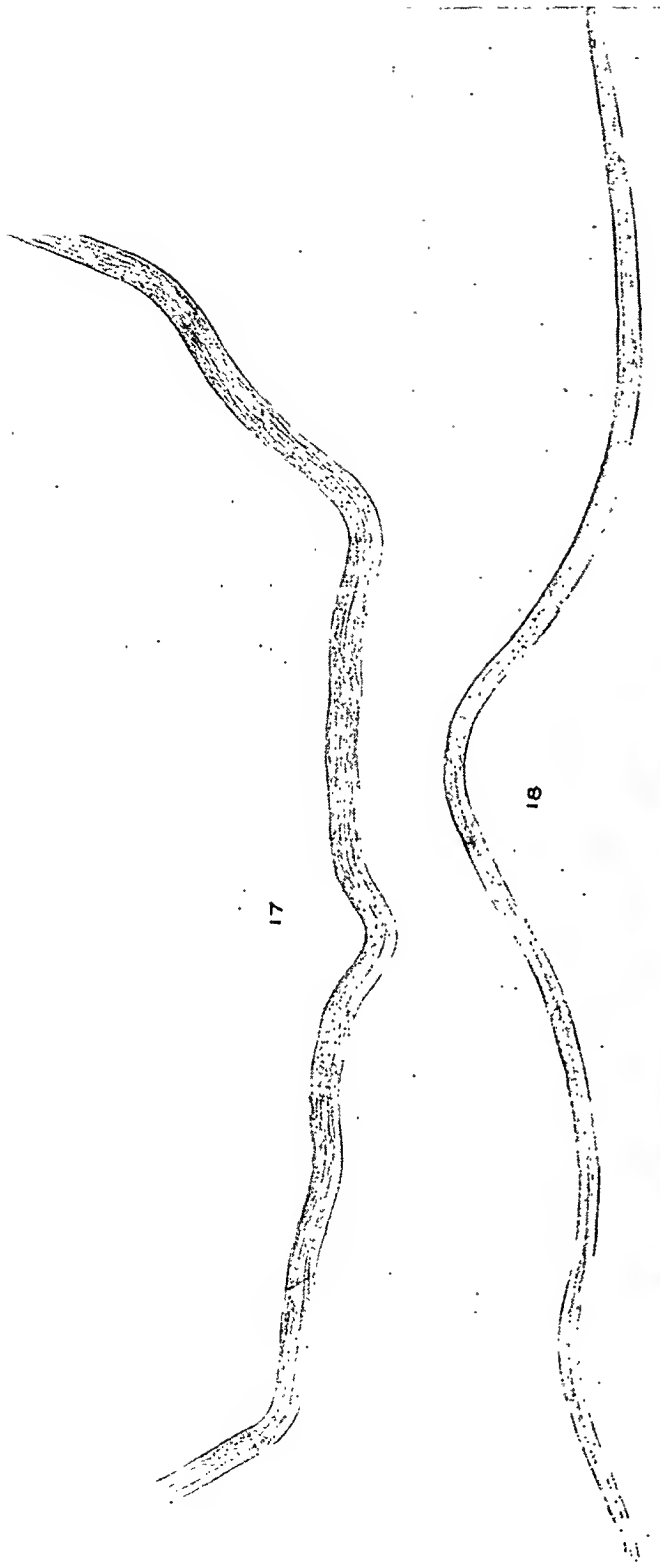


PLATE 2

THE MOSQUITOES AND MOSQUITO-BORNE DISEASES OF THE TREASURY ISLANDS¹ (BRITISH SOLOMON ISLANDS)

WILLIAM J. PERRY²

The fauna on many of the small islands in the Pacific Ocean was not well known prior to the onset of hostilities of World War II. Infrequent visits and scanty entomological surveys of these islands produced a wide break in continuity in our knowledge of the distribution of harmless insect, and disease-bearing vectors.

Detailed accounts were meager relative to the biological habits and medical relationships of important species of mosquitoes. Unseeded troops occupying military posts in endemic areas of such arthropod-borne diseases as malaria, dengue and filariasis became victims of disease due in part to our paucity of information on the "medical entomological" aspects of these South Pacific Islands.

The Treasury Islands represent one of the numerous important advance bases from which military thrusts were made against the Japanese in late 1943. Little was known relative to the presence and distribution of mosquito-borne diseases and no complete entomological records, with particular reference to mosquitoes, were obtained by earlier workers in the Solomon Islands.

Parasitological surveys conducted early in the campaign disclosed malaria to be of primary importance. The true malaria transmission rates, however, were masked by military personnel receiving suppressive therapy and an index of autochthonous malaria cannot be accurately given.

Prior to and during the military occupation, the native populace moved to Mono Island where a hyperendemic area was established in close proximity to the New Zealand forces. The greater contingent of American troops were quartered on Stirling Island and the apparent rise in readmission rates for April and May presented graphically in Figure 2 was due primarily to the removal of non-combatant troops from quinaerine therapy.

Dengue was not known to be endemic in the Treasury Islands and there were no outbreaks in the military forces during the occupation. The epidemiology of

¹ RESEARCH PROJECT NM 005 036. Acknowledgment. The author gratefully acknowledges the assistance and valuable suggestions of Commander J. S. Cowan, Commanding Officer, Naval Medical Field Research Laboratory, in the preparation of this project. The entomological assistance of Dr. L. E. Rozeboom of Johns Hopkins University, Dr. Allan Stone of the U. S. National Museum and Lieutenant Commander Nancy H. Wheeler of the Naval Medical School is deeply appreciated.

The opinions or conclusions contained in this article are those of the author and are not to be construed as necessarily reflecting the views or the endorsement of the Navy Department or the Naval Service at large.

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dengue in the Solomon Islands (1) appears to be dependent upon conditions that are favorable for the development and presence of *Aedes aegypti*. Although numerous opportunities were presented for the continued development of this species on the Treasury Islands, there were no introductions of *aegypti* to this area as late as 1945 (2). The species was not reported north of the Tulagi-Florida Islands.

Dissection studies on likely intermediate hosts, previously demonstrated by Byrd (3) and Schlosser (4) from the Solomons, indicated no fully developmental infective stage larvae of *Wuchereria bancrofti*. Blood smear studies on the native inhabitants of Falamai village on Mono Island revealed a 0.7 per cent infectivity rate for this pathogenic microfilaria.

Although no intermediary hosts revealing infective stage larvae of *W. bancrofti* were recovered in parasitic surveys, *Anopheles farauti* was suspected of being the primary agent in the transmission of this microfilaria. This assumption was influenced by: (1) epidemiology of the disease; (2) studies made by various authors in the South Pacific and (3) that *farauti* exhibited the highest incidence of developing larvae in the thoraces of the mosquitoes dissected.

The mosquito fauna was completely unknown, however, as on many of the coral and volcanic islands to the south, propagation of regional species was often limited by the existing physiographical characteristics of the islands. Several important topographical features affected the development of anophelines and culicines on the Treasury Islands. Among those features to be added to the brief summary given by Harper *et al.* (5) from this area were:

(1) Mono and Stirling were densely wooded and shaded. Typical breeding places as reported by Perry (6, 7) in the New Hebrides-Solomon Islands were not abundant.

(2) Frequent and heavy rainfall flushed the many streams which emptied abruptly into the bay from Mono Island. Flooding of coastal beaches was rare and river and stream outlets were seldom blocked by tidal action due to the protection afforded the southern coast by Blanche Harbour extending between Mono and Stirling Islands.

The results produced by these physiographical features are in marked contrast to the coastal lagoons formed by winding streams, with low gradients, flooding the extensive flat alluvial plains of Guadalcanal (8). Sand bars formed by the pounding action of the surf at stream and river outlets likewise facilitated spread of flood waters over wide areas.

(3) The thin veneer of soil and humus covering a porous coral floor provided excellent drainage and natural percolation of surface water. The lack of natural streams and standing bodies of water was in part responsible for the absence of mosquitoes on Watson, Wilson and Siana Islands.

(4) Commercial coconut plantations were few, and limited clearing of forested areas was done. This was an important factor in preventing the infiltration of anophelines to virgin areas from focal points of heavy breeding.

During a six months' survey by Paullus and Perry, ten species of mosquitoes

representing five genera were collected from Mono and Stirling Islands. The fauna included the following species:

Aedes (Stegomyia) *albolineatus* Theo.

Aedes (Finlaya) *albilabris* Edw.

Aedes (Stegomyia) *quasiscutellaris* F. & B.

Anopheles (Myzomyia) *farauti* Lav.

Armigeres breinli Taylor

Culex (Culex) *annulirostris* Skuse.

Culex (Lophoceratomyia) *fraudatrix* Theo.

Culex (Lutzia) *halifaxi* Theo.

Culex (Culex) *sitiens* Wied.

Tripteroides (Mimeteomyia) *solomonis* Edw.

No new species endogenous to the Treasury Islands were discovered when the known species were subsequently compared with those reported from Bougain-



FIG. 3. TYPICAL BREEDING PLACES OF *Anopheles farauti* IN SUNLIT AREAS AMONG MARGINAL DEBRIS OF STREAMS AND SWAMPS

ville to the immediate north and the New Georgia Islands to the south. The collections from Mono Island were limited to the southern coast and no records are available for elevations over 500 feet.

Anopheles (Myzomyia) *Farauti* Lav. Dense tropical forests covered much of the Treasury Islands and breeding of *Anopheles farauti* on Stirling was limited to the perimeter of Soala lake and the exposed margins of Soala stream.

With the onset of clearing and logging operations coincident with shore establishments, *farauti* extended its range to occupy the man-made breeding catchments. On Mono Island, adult females were collected in native huts and larvae were reported along stream margins within $\frac{1}{2}$ mile of Falamai village.

The larvae of *Anopheles farauti* exhibited constant morphological characteristics different from those reported by various authors (9) (10) in the species found in the New Hebrides-Solomon Islands. There was considerable variation in the degree of affinity of the bases in the prothoracic submedian hairs of the larvae. Clypeal hair 2 and head hairs 5, 6, and 7 were lightly branched. Hair 1 on abdominal segment 1 does not possess the palmate shape of the *farauti* larva in the New Hebrides and is definitely not hairlike as in *koliensis* from Guadalcanal (8).

Specimens of *farauti* from the Treasury Islands were examined by Dr. L. E.



FIG. 4. IMPORTANT SECONDARY, MAN-MADE BREEDING CATCHMENTS FOR AQUATIC STAGES OF *Anopheles farauti*

Rozeboom of Johns Hopkins University. In spite of the variations from his comparative studies of *farauti* in the Solomon Islands, he concluded that the larvae were definitely those of *farauti*. "The difficulty in positive identification of the *farauti* larva lies in its similarity to that of *koliensis* in New Guinea and other western areas . . . where the palmate (hair) of the first abdominal segment (of fourth instar larvae) may look like that of *koliensis* in being hairlike." Flattened elements of the palmate hairs, however, as shown by the specimens from the Treasury Islands are seldom seen in *koliensis*.

Culex (Culex) *Sitiens*. Numerous coral pockets found in the high cliffs of the south coast of Stirling Island supported water for mosquito breeding. *Culex sitiens* was the predominant form collected. This species is characteristically coastal in distribution and is well known for its ability to develop in brackish and salt water. It is rarely taken in collections of water inland from the coast. In a personal communication with Lever (1945) larvae were reported developing to adults in 16 days in water collected from Suva Bay in the Fiji Islands in which Marine cables were stored. Hurlbut (11) reported *sitiens* developing on Guam in 25 per cent sea water and that it developed less successfully in water of lower salt concentrations.

The Australasian records of *Culex sitiens* are considered to belong to *Culex jepsoni* (12, 13). The exact status of *sitiens* is still uncertain. Dr. Allan Stone of the U. S. National Museum tends to favor *sitiens* as the bona fide species. Likewise, Australian and British workers as Lever, Taylor and Lee refer to their Australasian records of this coastal brackish water breeder as *sitiens*.

The wide range exhibited by this species may explain the variations encountered in the aquatic forms. On this basis, as a partial explanation of morphological differences, and in view of the strict, limited breeding preferences of the species, *sitiens* is retained as the species from the Treasury Islands. Further studies involving a longer series of specimens from the known geographic range are necessary before an accurate evaluation of *sitiens* can be made.

In the several preserved specimens from the Treasury Islands the anal gills of the 4th instar larvae were somewhat longer than broad. The available specimens from these islands do not exhibit the short, bud-like gills as found in the larvae of *sitiens* from New Caledonia or the New Hebrides although they are still remarkable in being short, blunt and somewhat bulbous.

It has been noted that mounted specimens often exhibit variations in the shape of these structures when compared with freshly killed larvae. Since no specimens were available for study at the time an examination was made of the mosquito fauna from this area, it is not possible to ascertain the natural shape of these structures, and the gills are diagramed from preserved and mounted specimens.

Culex (Lutzia) *Hakifaxi* Theo. This large species was commonly collected in artificial containers and occasionally in the rock pools on the coral cliffs of Stirling Island.

The mouth brushes are adapted for use as accessory grasping organs, and the larvae are predatory on the aquatic forms of other species (14).

Culex (Culex) *Annulirostris* Skuse. *Culex annulirostris* is capable of utilizing many different types of catchments for breeding. Larvae and pupae have been collected in ground-pools, road ruts, hoof-prints and hog-wallows containing fresh, brackish or foul water. This species is widely distributed throughout the Solomon Islands and represents a common species in entomological collections. In spite of its extensive range and ability to utilize many types of water catchments for development, *annulirostris* from the Treasury Islands compares favorably with the redescription given by Buxton and Hopkins from Samoa (15).

The adult females are persistent biters in the early evening and morning hours. Byrd *et al.* (16) infected *annulirostris* in Samoa and demonstrated the filariae of *Wuchereria banerofti* fifteen days following an infective blood meal, however, no infective stage larvae were recovered.

Culex (Lophoceratomyia) *Fraudatrix* Theo. Lee (17) and Edwards (18) emphasize the frequent occurrence of a dark band beyond the mid-point of the siphon as an identifiable character for this species. This characteristic was not observed in many specimens collected and identified as *fraudatrix*, and it is felt that specimens tend to lose this pigmented band when preserved. It was rarely seen by the author on a long series examined from the New Hebrides.

The larvae were not abundant and the few specimens collected were from shallow pot-holes containing fresh water.

Tripteroides (Mimeteomyia) *Solomonis* Edw. The larva of this species was found primarily in water held in coconut shells and in occasional coral pockets containing high concentrations of dissolved organic tannins. The aquatic forms are not infrequently taken in association with *Armigeres breinli*.

Armigeres Breinli Taylor. Although the several known species are somewhat similar in structure, *breinli* is characterized by: large oval gills, short dark siphon, and absence of pecten teeth. A remarkable feature in the environmental habitats of *breinli* in the Treasury Islands is its ability to survive in stagnant water retained in putrifying coconut husks and hollow trunks of the bamboo and the sago palm.

The adults are vicious biters during the day, particularly in close proximity to breeding sites in the semi-mangrove scrub along the coast.

Aedes (Finlaya) *Albilabris* Edw. Larvae are abundant in tree holes and coral pools in which there has been an accumulation of organic matter. The larvae are striking in appearance due to the unusual stoutness of the clypeal spines.

Aedes (Finlaya) *Albolineatus* Theo. This species bears a close resemblance to *A. notoscriptus* and *A. albilabris*. The larvae breed by preference in water held in tree holes, coconut shells, tin cans and coral pockets.

Aedes (Stegomyia) *Quasiscutellaris* F. and B. The habitats occupied by the aquatic stages of *quasiscutellaris* are not unlike those of other members of the "scutellaris" complex throughout the Pacific Ocean area. Larvae have been recorded from all types of artificial containers and they have been collected on the Treasury Islands in tin cans, tree holes, and in coral pockets containing water high in organic matter such as decaying palm fronds, leaves, twigs and bread-fruit.

The larvae of the "scutellaris" complex of mosquitoes are not completely identified and further systematic studies are necessary before an evaluation of these closely related species can be made. The form illustrated from the Treasury Islands was drawn from a cast skin of a 4th instar larva, the adult of which was identified as *quasiscutellaris*.

This species has a known distribution throughout the Solomon Islands (19).

KEY TO THE 4TH INSTAR LARVAE OF THE TREASURY ISLANDS

1. Air tube lacking; palmate hairs present on abdomen. Tribe *Anophelini*.
Anopheles farauti Lav.
 Air tube present; abdomen without palmate hairs. Tribe *Culicini*. 2
2. Thorax and abdomen with rosettes of leaf-like setae. 4
 Thorax and abdomen without rosettes of leaf-like setae. 3
3. Pecten teeth extending entire length of air tube; mouth brushes with saw tooth projections. *Culex halifaxi* Theo.
 Pecten teeth lacking, or if present confined to basal portions of air tube; mouth brushes of normal shape. 5
4. Air tube with several multiple tufts of hairs extending length of tube; comb scales borne on a chitinized plate. *Tripteroides solomonis* Edw.
 Air tube with a single tuft of hairs following pecten teeth; comb scales borne singly, chitinized plate absent. *Aedes albolineatus* Theo.
5. Air tube without pecten teeth. *Armigeres breinli* Taylor
 Air tube with pecten teeth. 6
6. Air tube with a single tuft of hairs following pecten teeth. 7
 Air tube with 4 or more tufts of hairs following pecten teeth. 8
7. Comb scales arranged in a single row, individual scales pointed and fringed basally. *Aedes quasiseutellaris* F. & B.
 Comb scales arranged in an irregular patch; individual scales broad and fringed around entire margin. *Aedes albilabris* Edw.
8. Air tube with 4 tufts of multiple hairs. *Culex fraudatrix* Theo.
 Air tube with 5 or more tufts of multiple hairs. 9
9. Anal gills short and somewhat bulbous; one pair of subdorsal hair tufts on air tube. *Culex sitiens* Wied.
 Anal gills long and tapered; air tube without subdorsal hair tuft.
Culex annulirostris Skuse.

SUMMARY AND CONCLUSIONS

1. Ten species of mosquitoes representing five genera: *Anopheles*, *Aedes*, *Culex*, *Armigeres*, and *Tripteroides* were discovered on the Treasury Islands.

2. Of the mosquito-borne diseases known to be endemic in the South Pacific, malaria was of primary importance on Stirling and particularly Mono Island in the Treasury group.

3. Dengue has never been reported, and a 0.7 per cent infectivity rate for *Wuchereria bancrofti* was reported in the native inhabitants of Falamai village on the south coast of Mono Island.

4. A key to the fourth instar larvae is given and a brief discussion relative to the taxonomy and biology of the reported species is presented.

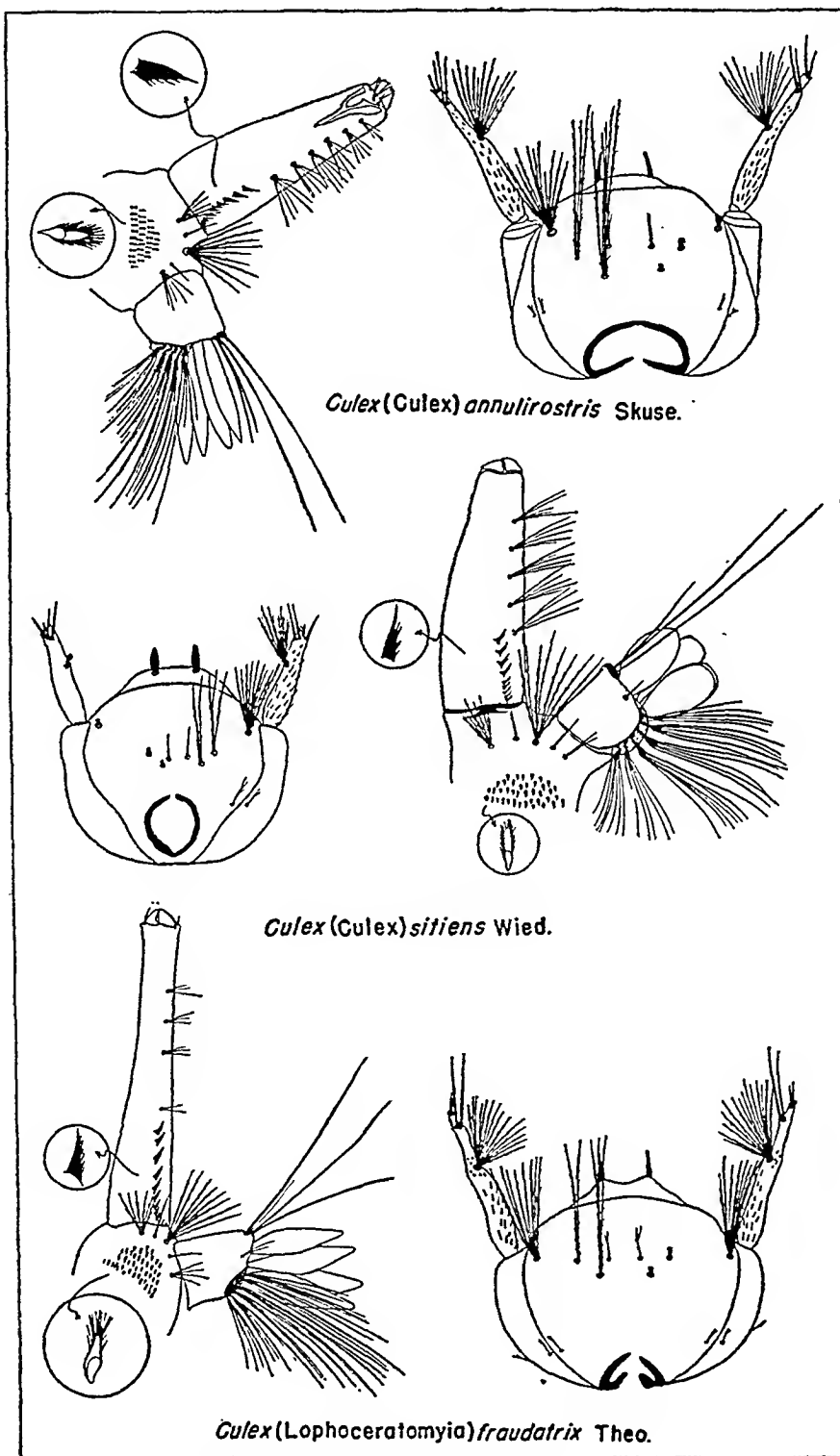


FIG. 5.

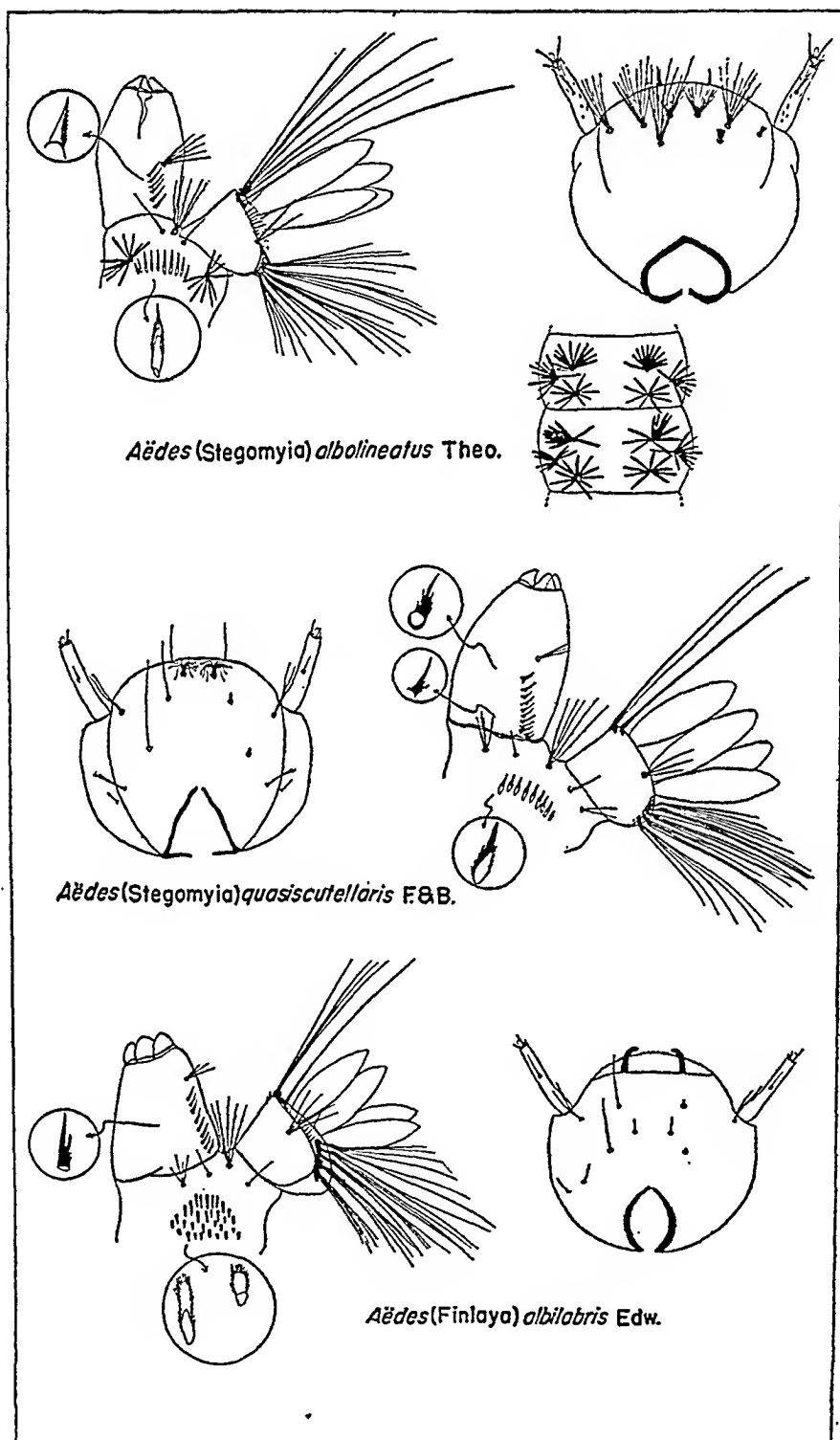


FIG. 6.

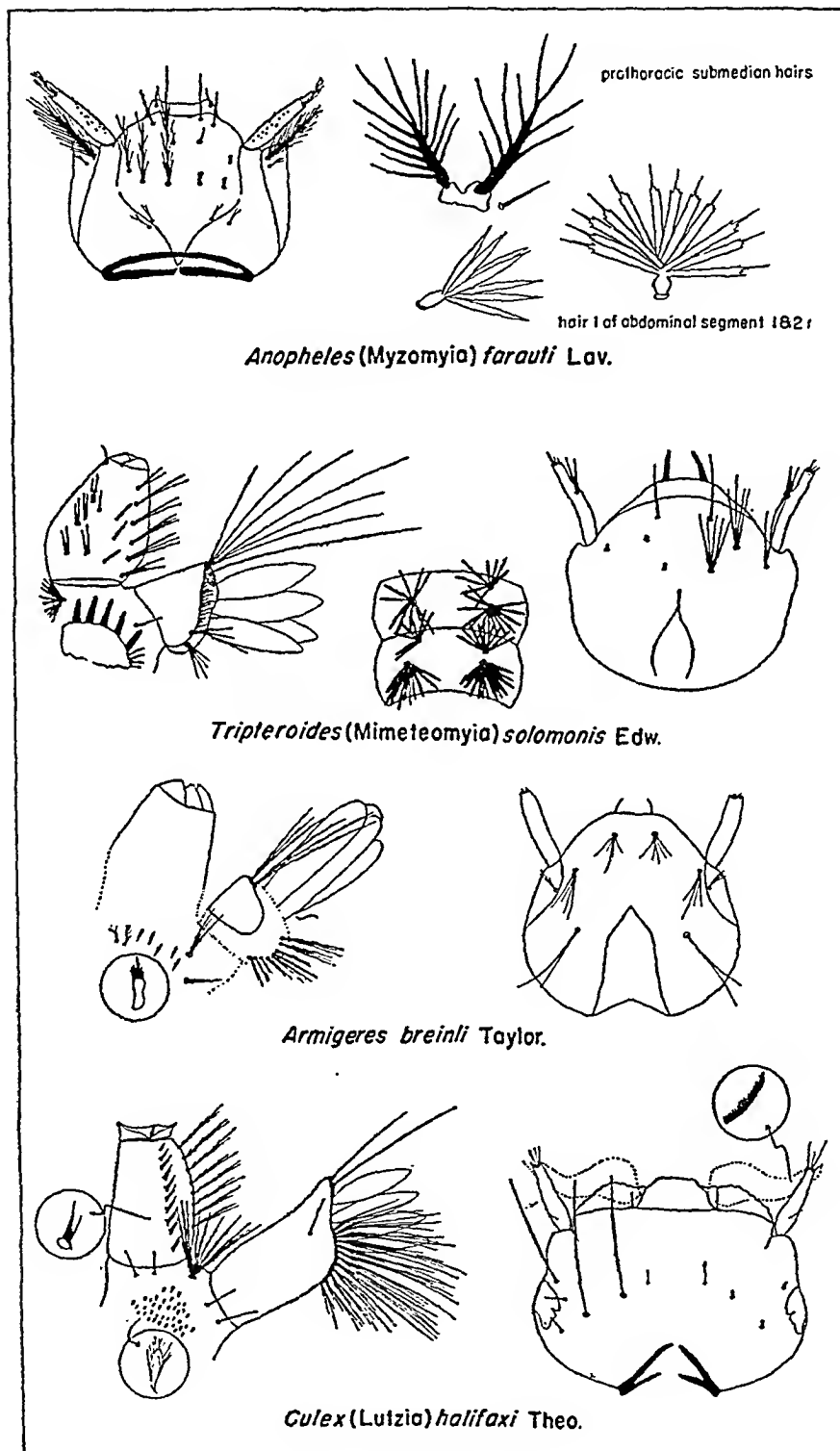


FIG. 7.

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THE BIOLOGY OF *LIPONYSSUS BACOTI* (HIRST, 1913) (ACARINA, LIPONYSSIDAE)¹

PETER SKALIY, BIOLOGIST, AND WAYLAND J. HAYES, JR., SR. ASS'T SURGEON

The present study was undertaken to develop a dependable technique for the investigation of the alleged rôle of *Liponyssus bacoti* in the transmission of murine typhus (1) and to extend our knowledge of its biology and ecology should the rôle of the mite prove important. Besides murine typhus, a variety of other diseases of man and animals have been associated with *L. bacoti* or with closely related mites, though in some instances the details and significance of the association are not clear. Many authors, (2, 3, 4, 5, 6, 7) have observed dermatitis in man resulting from the bites of rodent mites or from allergy due to them. Pulmonary infestation of man and monkeys by *Pneumonyssus* or related genera has been reported (8, 9). The filarial worm of the cotton rat, *Litomosoides carinii*, which has been the subject of numerous recent studies, is transmitted by *L. bacoti* (10, 11). Rickettsialpox is thought to be transmitted from mouse to mouse and from mouse to man by the mite, *Allodermanyssus sanguineus* (12), while *L. bacoti* has been shown capable of transmitting it from mouse to mouse in the laboratory (13). The chicken mite, *Dermanyssus gallinae*, is held responsible for perpetuating Western-equine encephalitis and the St. Louis virus among chickens (14, 15), while the Western-equine virus has been recovered from *L. sylviarum* and perhaps *D. americanus* taken from wild birds (16). A very interesting mixture of both viruses has been recovered from *L. sylviarum* (17). Congenital transmission of the St. Louis virus in the chicken mite has been reported (18, 19). The relationship of mites and the virus encephalitides has been adequately reviewed by Hammon (20). Hemorrhagic septicemia in snakes may be transmitted by *Ophinyssus serpentinum* (21). The hemogregarine of rats, *Hepatozoon perniciosum*, is transmitted by *Laelaps echidninus* (22). Much more distantly related are the scabies and follicle mites, the pentastomes, the chiggers associated with dermatitis and with the transmission of tsutsugamushi (23, 24, 25), and the orbatid mites responsible for the transmission of various anoplocephaline cestodes of mammals (26). The possibility has been discussed that several other diseases, including plague, for which the usual mode of transmission is well-known may occasionally be transmitted by mites.

Most of the methods developed for rearing *L. bacoti* in the laboratory consist essentially of providing the mites with rats and litter and thus simulating the conditions under which they live in nature (27, 28). They lack, however, the precision necessary for a refined study of either disease transmission or biology. In 1946, Bertram, Unsworth, and Gordon (29) described a procedure by which *L. bacoti* could be reared in tubes except during the protonymph stage of the life cycle. This technique enabled these authors to add many details to our knowledge of the biology of the mite and to make more exacting studies of its

¹ From the Technical Development Division, Savannah, Georgia. Communicable Disease Center, U. S. Public Health Service, Atlanta, Georgia.

rôle in the transmission of *Litomosoides carinii*. Using a modification of this technique, it has been possible to rear mites through the entire life cycle within tubes where they are constantly available for observation.

It has been necessary to partially restate certain introductory material already adequately covered by Bertram and his collaborators. However, their excellent paper must be consulted by anyone interested in the behavior and morphology of the various stages and, of course, in mite-borne rat filariasis. Their bibliography adequately covers the taxonomy of the form. This present study contributes to the knowledge of: (1) the effect of temperature and humidity, (2) the duration of adult life, (3) egg production, and (4) parthenogenesis.

TABLE 1

Measurements of living Liponyssus bacoti in various stages of development

Unfed protonymphs had no blood meal. Unfed adults did not show a recent blood meal. Only those maximally extended were considered engorged.

STAGE AND DEGREE OF FEEDING	NUMBER OBSERVED	AVERAGE MM.		STANDARD DEVIATION ± MM.	
		Length	Width	Length	Width
Eggs.....	41	0.324	0.206	0.043	0.025
Larvae.....	25	0.539	0.220	0.067	0.024
Unfed Protonymphs.....	37	0.618	0.206	0.089	0.036
Fed Protonymphs.....	20	0.659	0.273	0.132	0.068
Unfed Adults ♂.....	28	0.764	0.288	0.144	0.059
Fed Adults ♂.....	11	0.890	0.318	0.195	0.094
Unfed Adults ♀.....	21	1.153	0.459	0.101	0.067
Fed Adults ♀.....	47	1.292	0.578	0.329	0.130
Engorged Adults ♀.....	14	1.406	0.575	0.195	0.109

MATERIALS AND METHODS

The mites used in this study belong to a strain collected from *Rattus norvegicus* in Savannah, Ga., and maintained in the laboratory since September 1946. Measurements of the body size of living mites of this strain are shown in Table 1. Protonymphs listed as fed had one or more blood meals but were not necessarily completely engorged. No segregation as to sex was made for protonymphs. Adults were considered fed when a bright red coloration of the gut was observed, while only those which seemed maximally extended were considered engorged. Unfed adults did not have a red coloration, indicating no recent blood meal.

For the present biological studies, the mites were reared in 13 x 100 mm. pyrex culture tubes (Wassermann tubes). The tubes were stoppered with non-absorbent black cotton covered by a single layer of black nylon netting. Each tube was provided with a strip of filter paper dyed black with household dye. The strips were 1½ inches long and just wide enough to fit snugly in the tube. Before being placed in the tube, the surface of these strips was scratched lightly. This provided a roughened surface for mites to crawl in but prevented their burrowing entirely into the paper and thus escaping observation.

Mites were maintained in the tubes continuously throughout their cycle and fed on a rat's tail introduced into the tube. The rat was held in a special metal tube of appropriate size provided with a grating at the head end to permit breathing. At the other end, the tube was provided with a lid having a single opening through which the rat's tail extended. Rats of various ages were used. No adverse effects on the health of rats were observed. Although several sizes of rat tubes were available, it was sometimes necessary to loop a piece of adhesive tape around the proximal part of the tail and secure the ends of the tape to the sides of the rat tube in order to prevent the rat from withdrawing its tail into the tube. After the rat was properly held, its tail was washed, shaved, and the dorsum scraped for a length of about 1 inch with a razor blade held at right angles to the axis of the tail. The scraping removed the scales and left a delicate membrane which did not bleed but through which protonymphs could feed. Adults can feed successfully, though somewhat slowly, on the unprepared tail; protonymphs require more tender skin on which to feed and normally find it on the more delicate parts of the body. However, contrary to the experience of Bertram and his collaborators (29), we found that protonymphs fed readily on the prepared tail. This had the great advantage of allowing the continuous observation of an entire generation of mites, the identity of which remained known throughout the experiment. After the rat's tail was prepared, a heavy ring of plasticine was molded around the proximal part. The culture tube was then passed over the tail and embedded in the plasticine which held the tube firmly and acted as a plug. Immediately after the rat's tail was inserted into the tube, some mites left the filter paper and began feeding. Such mites often finished feeding within 5 to 15 minutes, and one protonymph was observed to complete feeding within 1 minute. However, some mites, presumably less hungry than the others or finding it more difficult to locate a suitable feeding surface, did not begin to feed at once. In practice, the tube of mites was left on the rat's tail for 1 hour daily when there were a considerable number of mites in the tube, and it is probable that not all of them fed on any one day. When only a few mites were present, they were removed from the rat's tail as soon as all were observed to be completely fed and, at favorable temperatures and humidities, not returned until the 3rd day by which time the first blood meal would be largely digested. On the other hand, at temperatures unfavorable to feeding, the feeding period was extended to as much as 5 hours daily to offer the best possible opportunity for survival.

After the mites had fed, most of them returned to the strip of filter paper. The few mites which remained on the tail were brushed off with a camel hair brush into a pan of water. Mites were then removed from the surface film of water by a very fine wire (0.008-inch diameter) formed into a loop 2 mm. in diameter. When the loop carrying a mite was touched to filter paper, the water was absorbed and the mite walked away.

It often happened that, because of secretions and the oozing of serum from the rat's tail, the inside of the culture tube became moist during feeding. If mites are trapped in a film of moisture, they will die. This difficulty was overcome by

drying the culture tubes with a gentle stream of air from an ordinary compressed air source, conducted through a drying tube of calcium chloride until all visible moisture was gone. The air entered through one opening of a 2-hole rubber stopper (size 00) and passed out of the tube through the 2nd hole, which was covered by a fine mesh to prevent escape of the other mites.

Artificial methods of feeding mites were investigated in an effort to develop an even more simple method for studying their biology and, especially, to facilitate the use of controlled infecting blood meals in transmission studies. The simplest artificial feeding technique consists of placing a drop of blood, or, better, a small piece of natural sponge soaked in blood, directly into the tube with the mites. This method was entirely successful for *Laelaps echidninus* which fed promptly and completely on unwarmed rat heart blood treated with either sodium citrate or heparin. The technique was entirely unsuccessful for *L. bacoti*. Unsuccessful also were attempts to induce both species of mites to feed through membranes of paper, Cellophane,² or capping membrane³ placed over carefully warmed metal or glass tubes containing blood. Apparently an adequate stimulus to feeding for *Laelaps echidninus* is the odor or taste of blood; what constitutes an adequate stimulus for *L. bacoti* is not clear.

Eggs were collected by placing 50 or more well-engorged adult females in a culture tube. The mites preferred the texture of roughened filter paper to that of glass or nylon and laid most of their eggs on the paper. Egg laying was usually allowed to continue for 24 hours but was stopped at the end of 1 hour for special studies. At the end of the desired period, the strip of filter paper was removed and the adults were brushed off into a pan of water. The undisturbed eggs on the strip of filter paper were then placed in a fresh culture tube. The age of each generation was thus known within exact limits. The 24-hour period during which an egg was laid was counted as the first day; the 24-hour period during which the deutonymph moulted was counted as the last day in the egg-to-adult cycle.

When it was necessary to move mites in the larval, nymphal, or adult stage from one tube to another, the old culture tube was attached to a fresh one mouth-to-mouth by a strip of cellulose tape. The mites then were driven slowly from one tube to the other by a pre-heated metal ring which fitted closely the diameter of the tubes. When it was necessary to have ready access with dissecting needles and forceps to the inside of a culture tube for an extended period, the mites were prevented from escaping by an electrically heated ring. The ring was made double so that it would hold either one or two tubes. It consisted of a copper base around which was wound 18 inches of insulated heating coil consisting of fine resistance wire on a core and offering 100 ohms of resistance per inch of coil. Such insulated coils may be obtained at any radio appliance shop. The ring consumed approximately nine watts and could be used directly on a 110-volt line.

The majority of tests were carried out in an air-conditioned laboratory where both temperature and relative humidity were controlled. Conditions other than

² A product of E. I. duPont deNemours, Co., Wilmington, Delaware.

³ A product of E. Troeder, Bellville, New Jersey.

those of the laboratory were obtained by use of incubators and refrigerators provided with controls for regulating the temperature and with constant relative humidity devices. Variations in temperature and relative humidity for the various conditions studied are shown in Table 2. The various ranges of temperature and relative humidity studied are shown in the first two columns while the extreme variations from those ranges are shown in the columns to the right. The greatest extremes occurred at laboratory conditions; however, these were of relatively short duration as shown by a hygrothermograph: 7.2 degrees above 26°C. was recorded for 2 hours, 11° below 24°C. for 2 hours, 51 per cent above 47 per cent for 6 hours, and 19 per cent below 47 per cent for less than 1 hour during the

TABLE 2

Variations of temperature and relative humidity for the different conditions studied

Values are extremes recorded during entire period.

TEMPERATURE	RELATIVE HUMIDITY	TEMPERATURE VARIATIONS		RELATIVE HUMIDITY VARIATIONS	
		Above Range	Below Range	Above Range	Below Range
°C.	%	°C.		%	
6-8	41-42	3.0	0.0	33	33
18-20	39-44	0.3	0.3	24	16
24-26	18-20	3.4	0.7	3	2
24-26	23-24	1.2	0.0	2	3
24-26	47	7.2*	11.0*	51*	19*
24-26	100	0.1	2.2	0	0
30-32	20	0.0	0.0	4	3
30-32	69-70	0.8	0.0	4	7
32-34	68-69	0.0	0.6	3	7
32-34	100	0.6	0.6	0	0
34-36	31-32	0.0	0.0	3	1
34-36	39-40	0.0	0.0	2	0
34-36	58-60	0.5	0.0	3	0
34-36	64-65	0.0	0.0	4	3
34-36	100	0.0	0.0	0	0
36-38	100	1.0	0.0	0	0

* For the duration of these extremes see text.

entire 37 weeks of study. Variations for conditions other than those of the laboratory were of smaller magnitude and shorter duration.

LIFE CYCLE

The life cycle observed during these studies conforms with that reported by Bertram, Unsworth, and Gordon (29). It consists of the following stages:

1. The blood-sucking adult male and female
2. The egg
3. The hexapod non-feeding larva
4. The octopod blood-sucking protonymph
5. The octopod non-feeding deutonymph.

Each immature stage moults only once, while the adult does no moulting. One complete blood engorgement is necessary for the protonymph and one for the adult to finish the cycle. This engorgement, in the case of the protonymph, is usually achieved only after two or more feedings although a few protonymphs have been observed to engorge at the first feeding. The majority of adults engorge during the first feeding after adulthood is reached, while a few require two feedings to accomplish this. Following the first engorgement as an adult, the female produces its first eggs, completing the cycle. The shortest egg-to-egg cycle observed has been 13 days. Bertram and his collaborators observed that 10 to 12 days were required for completion of the life cycle. These figures are consistent

TABLE 3

Per cent mortality of Liponyssus bacoti exposed to different temperatures and relative humidities

Mortality during each stage is based on the number of individuals entering the indicated instar, and the total mortality during development is based on the original number of eggs.

TEMPERATURE	RELATIVE HUMIDITY	NUMBER OF EGGS	STAGE MORTALITY				TOTAL MORTALITY
			Egg	Larva	Proto.	Deuto.	
°C.	%		%				%
6-8	41-42	0	No eggs laid				
18-20	39-44	36	13.9	3.2	46.7	6.3	58.3
24-26	18-20	125	75.2	0.0	51.6	6.7	88.8
24-26	23-24	145	94.5	0.0	50.0	0.0	97.2
24-26	47	627	11.8	2.2	27.0	0.8	37.5
24-26	100	67	3.0	0.0	63.1	0.0	64.2
30-32	20	27	100.0	—	—	—	100.0
30-32	69-70	28	0.0	0.0	67.9	0.0	67.8
32-34	68-69	28	7.1	0.0	61.5	10.0	67.8
32-34	100	82	1.2	0.0	84.0	0.0	84.1
34-36	31-32	141	99.3	100.0	—	—	100.0
34-36	39-40	144	96.5	20.0	100.0	—	100.0
34-36	58-60	148	83.3	4.2	100.0	—	100.0
34-36	64-65	350	52.0	0.6	85.6	29.2	95.1
34-36	100	83	28.8	11.9	88.5	16.7	94.0
36-38	100	223	78.5	100.0	—	—	100.0

since Bertram allowed protonymphs free access to the rat and probably, therefore, a slightly better opportunity for rapid development.

RESULTS

The success with which mites were reared under various conditions is shown in Table 3. Each horizontal line represents the number of eggs studied at each particular combination of temperature and relative humidity and the per cent mortality during each stage of the ensuing development. The per cent mortality for each stage is based on the number of mites actually entering that stage, while the total mortality during development is based on the original number of eggs. Expressed in this way, the total mortality is not always exactly equal to

the sum of the figures for the separate stages. In each instance, unless specifically stated, the eggs were laid under the same conditions of temperature and relative humidity as those under which their subsequent development was observed.

The highest percentage of eggs developed into adults under conditions in the open laboratory (average of 25°C. and 47 per cent relative humidity). Under these conditions (see Table 3) the mortality of eggs was 11.8 per cent, and that of larvae only 2.2 per cent. The greatest mortality of immature mites, 27.0 per cent, occurred during the protonymph stage. Whether this was due to faults in technique or is natural and due to the greater vicissitudes endured by the protonymph or to some intrinsic weakness is not clear. The lowest mortality occurred during the deutonymph stage; only 0.8 per cent failed to develop into adults. For the entire cycle, egg-to-adult, the total mortality based on 627 eggs was 37.5 per cent or a survival of 62.5 per cent.

TABLE 4

Duration of egg stage at different temperatures and relative humidities

Twenty-four-hour period during which batch of eggs was laid was counted as first day.

TEMPERATURE	RELATIVE HUMIDITY	NUMBER OF EGGS OBSERVED	NUMBER OF EGGS DIED	NUMBER OF EGGS HATCHING ON INDICATED DAYS							
				1	2	3	4	5	6	7-13	13-25
°C.	%			No reproduction							
6-8	41-42	0	0	0	0	0	0	0	0	6	22
12	100	45	17	0	62	31	1	0	0	0	0
24-26	47	107	13	0	3	58	0	0	0	0	0
24-26	100	63	2	0	7	19	0	0	0	0	0
32-34	68-69	28	2	0	72	7	0	0	0	0	0
32-34	100	80	1	0	25	0	0	0	0	0	0
34-36	58-60	134	109	0	95	4	0	0	0	0	0
34-36	100	143	31	13	14	10	12	0	0	0	0
36-38	100	155	119	0							

Table 4 shows the duration of the egg stage under various conditions of temperature and relative humidity. For 107 eggs observed under laboratory conditions, the egg stage required from 2 to 4 days. The greatest number of eggs hatched on the 2nd day. The larval stage, in all mites, was approximately 24 hours in length. Due to the necessity of feeding in the protonymph stage, the length of time required for the mite to moult may have been influenced by the frequency of feeding and the time permitted for feeding. The shortest duration of the protonymph stage was observed to be 3 days; the longest 28 days. In each case, only one mite was concerned. Of 307 protonymphs observed, 95.1 per cent moulted within 5 to 14 days after casting their larval skin. Unfed protonymphs did not moult; the duration of the stage in these mites was determined by death. Protonymphs exposed to a temperature of 25°C. and 47 per cent relative humidity died within 7 to 12 days; by increasing the relative humidity to 100 per cent, death was delayed until at least the 23rd day and in one mite until the 46th day. The majority (64.3 per cent) of unfed protonymphs held at 100 per cent relative

humidity died between the 27th and 32nd day. In comparison to the protonymph, the deutonymph is relatively inactive. Within 24 to 48 hours after becoming a deutonymph, the skin is shed and the mite becomes an adult male or female.

Table 5 shows the length of the total development period at various combinations of temperature and relative humidity. For 305 adults whose development was observed in the open laboratory, the shortest cycle egg-to-adult was 7 days; the longest, 34 days. The majority of mites (78.7 per cent) required from 11 to 16 days.

The duration of the adult period in the female and the total egg production are shown in Table 6. Twenty-six adult females were studied. The shortest period of survival was 21 days, the longest 90, and the average for all females, 61.9 days.

TABLE 5

Percentage of mites reaching the adult stage after different numbers of days when exposed to different temperatures and relative humidities

Twenty-four-hour period during which egg was laid counted as the first day. Twenty-four-hour period during which deutonymph moulted counted as the last day.

TEMP.	R. H.*	NO. OF ADULTS	PER CENT OF MITES REACHING ADULT STAGE IN INDICATED NUMBER OF DAYS																			
			7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	over 24	
°C.	%																					
18-20	39-44	15						6.6			46.6					40.0	6.6					
24-26	18-20	14					92.9			7.1												
24-26	23-24	4					25.0			25.0	25.0			25.0								
24-26	47	305	0.3	1.3	1.6	3.6	8.5	18.4	9.5	13.1	11.5	17.7	1.3	2.3	5.6	1.0	0.7	2.0	1.0		0.6	
24-26	100	19									10.6	15.3	10.6			31.6	10.6	10.6		5.3	5.3	
30-32	69-70	9							11.1	11.1	11.1			11.1								
32-34	68-69	9				22.2			22.2					11.1								
32-34	100	13				23.1			15.4		7.8	7.8	7.8			33.3			11.1			
34-36	64-65	17			5.9			64.7	5.9	17.6					30.7	7.8						
34-36	100	6	66.6		16.6																	

* Relative Humidity.

In the study of egg production, each female was reared from an isolated egg in a separate tube and kept apart from other females during the adult period. Table 6 shows two groups of females; unfertilized females were isolated during the entire life period; those which were mated with adult males were listed as fertilized. Three male adults were placed in each tube with the latter group of females immediately following the first engorgement. There were two exceptions to this procedure: 2 males were placed with female 50D after the 4th engorgement and 6 with female 50Q after the 6th engorgement. The egg-laying period began 3 to 10 days after the female became adult and after 1 or 2 feedings. Of 26 females, 2 began laying within 5 days after becoming adults, 21 in 6 days, 1 in 7 days, and 2 in 10 days. The relative degree of engorgement of the female determined the time interval between feedings. Mites were exposed to the rat's tail as early as the 2nd or as late as the 7th day following an engorgement; if no feeding or only partial engorgement took place during a feeding opportunity, mites were fed on successive days until engorged. The largest number of engorge-

ments occurred at an interval of from 3 to 6 days. Egg-laying normally followed within 24 hours after each engorgement, the number of eggs varying from 1 to 20. Total eggs produced by individual females are shown in Table 6. The lowest egg production, which occurred in female 30A, was 8 eggs; the largest, in female 33E, 141 eggs. The mean of each group is shown in the table; that of both groups is 98.8.

The effects of increasing temperature and varying relative humidity on the rearing of mites are shown in Tables 3 and 5. Horizontal lines in the tables with temperatures of 30° to 32°C., 32° to 34°C., 34° to 36°C., and 36° to 38°C. represent the increased temperatures with different relative humidities. Of the 1,554

TABLE 6

Survival and egg production of female Liponyssus bacoti

First day of adult period was the twenty-four-hour period during which deutonymph moulted. Unfertilized females were reared in isolation; fertilized females were mated after becoming adult.

UNFERTILIZED			FERTILIZED		
Female	Adult Period Days	No. Eggs Produced	Female	Adult Period Days	No. Eggs Produced
33D	21	22	50C	29	33
30A	22	8	50H	46	99
29F2	48	82	71E	47	87
33J	52	132	45C	49	88
33K	52	99	60B	58	125
22F	53	68	71E	63	131
22E	64	110	45D	65	92
29F1	66	123	50M	71	120
33C	73	100	45O	72	88
37D	76	131	60A	72	112
41I	88	127	50Z	84	119
33E	90	141	50Z'	85	108
			50Q	86	99
			50D	86	124
Mean.....	58.7	95.3	Mean.....	65.2	101.8

eggs laid by adults and observed at these same temperature ranges, only 53 survived the nymphal stages and developed into adults.

Eggs produced at the higher temperatures generally show an increase in mortality, as compared to those laid and hatched in the open laboratory. Exceptions were noted at 30° to 32°C. with a relative humidity of 69 per cent and at 32° to 34°C. with a relative humidity at 68 and 100 per cent. There is some indication from these exceptions and the results obtained at 24° to 26°C. and 100 per cent relative humidity that an increase in relative humidity over that of the laboratory is favorable to the survival of the egg. Increasing temperatures also decreased the length of the egg stage except at 36° to 38°C. and 100 per cent humidity, under which conditions hatching appeared retarded, Table 4.

Higher temperatures also generally increased mortality during the larval stage except at 30° to 32° C. with 69 per cent relative humidity, 32° to 34° C. with 68 and 100 per cent relative humidities, and 34° to 36°C. with 64 per cent relative humidity. These exceptions gave mortalities less than the 2.2 per cent obtained in the open laboratory, but the difference cannot be shown to be significant on the basis of present data. The duration of the larval stage appeared to be shortened by the increased temperatures since a greater proportion of the mites moulted within 24 hours than under laboratory conditions. Moreover, at the higher temperatures, a greater proportion of larvae died in the process of emerging from the egg shell. Larvae exposed to 36° to 38°C. and 100 per cent relative humidity died in a distended condition, the body being filled with a clear fluid.

In the protonymph stage, it was found more difficult to feed the mites at 30°C. and above than under laboratory conditions. The more rapid accumulation of moisture on the scarified tail of the rat tended to make the surface unsuitable for feeding before the protonymphs had sufficient time to take a blood meal. Excess moisture also collected on the wall of the tube and trapped protonymphs moving about in search of a satisfactory feeding place. These factors may have delayed the engorgement of some protonymphs which did feed and may thus have increased the duration of the stage. Further, under open laboratory conditions, the insertion of the rat's tail into a tube activated protonymphs and usually resulted in their finding the tail and feeding. At higher temperatures, a similar response was not observed. It appeared that protonymphs did not as readily detect the opportunity to feed. A few protonymphs in a starved condition failed to feed, although they were given several opportunities of long duration on successive days with no excess moisture or other observable deterrent present. In general, it appeared that all protonymphs failed to feed as readily at the higher temperatures. All these difficulties increased the mortality at this stage and tended to increase the duration of the protonymph stage for those which survived.

Increased mortality was also observed in deutonymphs reared at the higher temperatures. Of the nine deutonymphs which failed to develop into adults, 5 died in the process of moulting, 1 died engulfed in moisture, and 3 died of unknown causes. The length of the deutonymph stage was decreased in some mites; the majority moulted within 24 to 48 hours, and a few emerged in less than 24 hours. The deutonymphs differed from those reared under normal laboratory conditions in the coloration of the distended gut. A black-red color in deutonymphs reared at higher temperatures as compared to a bright-red color in those reared under the usual laboratory environment indicated increased digestion of the blood meal at the higher temperature.

Lowering the temperature below that of the open laboratory increased the mortality and length of the various developmental stages. At 6-8°C. and an average relative humidity of 41 to 42 per cent, no egg production occurred. Approximately 100 healthy, young, well-fed adult females taken from an open culture in the insectary and exposed to these conditions died over a period of 38

days without producing a single egg. Approximately 50 similar females from the same source produced 86 eggs over a period of 96 hours, while exposed to a temperature of 13° to 13.3°C. and a relative humidity of 100 per cent. These eggs were then exposed to an average temperature of 12°C., and 100 per cent relative humidity, and only 45 (52.3 per cent) of the 86 eggs failed to develop into larvae. The egg stage required from 7 to 25 days under these conditions (Table 4.). At 12°C. average temperature, the mortality of larvae was 36.6 per cent as compared to 2.2 per cent under ordinary laboratory conditions. Under the same conditions, no larva developed into a protonymph for at least 5 days, while in the open laboratory the stage lasted only approximately 24 hours.

Of the small number of protonymphs studied at the lower temperatures, none developed beyond that stage. The failure of any protonymph to feed satisfactorily, even though opportunity was presented daily for 3 to 4 hours, prevented further development. Only one protonymph was observed to take a small blood meal, and subsequent feeding was not accomplished.

The occurrence of parthenogenesis resulting in only male offspring has been reported for *L. nagayoi* (30). The occurrence of parthenogenesis in *L. bacoti* has also been reported (29). Further study has confirmed this for *L. bacoti* and determined that, as in *L. nagayoi*, all the offspring are male.

Two females (29F1 and 29F2 in Table 6), reared from the egg in separate tubes produced 205 eggs, all of which were observed. One egg died; no larvae died; 42 protonymphs died or were lost; and 3 deutonymphs died. A total of 159 adults or 77.6 per cent emerged, but 5 were lost. The 154 remaining adults were cleared for microscopic study and the sex determined on the basis of morphology. They were all male. The survival rate of 77.6 per cent was slightly higher than the general average obtained under the same conditions of temperature and humidity, indicating that these parthenogenetically produced males were not weakened. Some parthenogenetically produced males were allowed access to a previously unfertilized isolated female which had already produced many eggs. Subsequent eggs of that female produced both male and female offspring showing that the parthenogenetically produced males were capable of normal function and also that the same female can produce parthenogenetic, and later, fertilized eggs. No experiment was conducted to test whether a female requires repeated insemination or only a single insemination in order to produce fertilized eggs throughout her life. No study of the cytology of parthenogenesis was attempted. The normal sex ratio was not established.

SUMMARY AND DISCUSSION

The list of diseases associated with mites and especially with the liponyssid mites has been reviewed.

A method for rearing and maintaining mites that permits the continuous observation of individual mites during their entire life has been described.

The observations on the effect of temperature and relative humidity appear to establish within rather narrow limits the conditions of the micro-environment under which the mite can multiply either in the laboratory or in nature. Since

the eggs are always laid away from the host, the natural environmental temperature determines their hatching. Egg laying, and therefore reproduction, does not occur at 6° to 8°C., although the adult may live for more than a month at these temperatures. Eggs are laid and protonymphs develop at 12° to 14°C. Protonymphs in nature feed on the body of the rat deep within the fur and protected from a cold environment. It appears likely that 12° to 14°C. represents the lowest temperature at which *L. bacoti* can reproduce, and then only with a great lengthening of the cycle and an increase in mortality.

At low and intermediate temperatures, these mites can tolerate a wide range of relative humidity. At 24° to 26°C., they withstood a relative humidity of 18 to 20 per cent. However, with increasing temperatures, increasing humidity is necessary to permit survival. Slightly over 60 per cent relative humidity is necessary to permit a complete cycle at 34° to 36°C., while even at 100 per cent relative humidity all mites died at 36° to 38°C.

At least for the temperatures and relative humidities studied, the conditions of the open laboratory (24° to 26°C. and 47 per cent relative humidity) gave the best over-all opportunity for development and permitted rearing to the adult stage of 62.5 per cent of some 627 eggs studied under those conditions. There was, however, some evidence that humidities higher than 47 per cent are more favorable to survival of the egg. In the open laboratory, the average life of the adult female was 61.9 days and the average number of eggs per female 98.8; a little over $\frac{3}{4}$ of the mites reached the adult stage between the 11th and the 16th day. These figures suggest a considerable reproductive ability in *L. bacoti* and, since they undoubtedly reflect some mortality due to handling, it may be that the mites reproduce even more efficiently in nature under favorable conditions.

Observations in January and February 1948 at Savannah showed the lowest temperature of even the accessible portion of both protected and unprotected *Rattus norvegicus* burrows was 7.2°C. while the highest temperature was 20.5°C. The relative humidity of these burrows was usually over 90 per cent but never as high as 100 per cent. Temperature changes in the burrows always lagged behind and were less marked than those of the environment. The average burrow temperature in winter was warmer than the environmental temperature. These observations suggest that, even in the coldest months of winter, many days occur in the Savannah climate which could permit some reproduction of *L. bacoti*.

The laboratory studies suggest some of the factors which may be expected to favor the appearance of excessive numbers of *L. bacoti* or to greatly reduce these ectoparasites in nature.

No significant difference occurred in the total egg production and in the length of the adult period of fertilized and unfertilized females. Unfertilized eggs developed parthenogenetically into male offspring which as adults, were capable of fertilizing female adults.

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PHLEBOTOMUS AND RESIDUAL DDT IN GREECE AND ITALY¹

MARSHALL HERTIG²

In a number of countries residual DDT applied in various ways has been shown to give consistently good results in the control of *Phlebotomus*. The treatment of the inner walls of living quarters in all reported instances (1, 2, 3) has given virtually complete protection from the bites of *Phlebotomus*, with usually no sandflies to be found in houses in daytime searches. A high degree of area control was secured in Peru by spraying outdoor stone walls as well as the interiors of houses (3). DDT was also used successfully against sandflies by various German investigators during the war (4). These various studies, however, did not determine the degree of area control which would be produced by house spraying alone. Furthermore there had been few long-term observations in the temperate zone where many of the most important sandfly-borne disease problems occur, and where theoretically a single annual pre-season treatment ought to yield a high degree of control. It has been obvious for some time that the now standard method of house spraying for malaria control is also and automatically a method for the control of *Phlebotomus*. It has so happened, however, that in the urgency of the malaria work only casual attention has been paid to the fate of *Phlebotomus* in various campaigns in regions where these two problems coexist.

In Greece for the past several years there has been carried out a very extensive malaria campaign. Houses in most of the villages of the country have received an annual treatment with DDT, followed by a spectacular decrease in malaria (5, 6). This project has been carried out by the Malaria Service of the Greek Government with the technical and financial assistance, first of UNRRA and later of the World Health Organization and the American Mission for Aid to Greece. It had not proved possible to make specific observations on *Phlebotomus* but general reports indicated that these normally abundant insects had greatly decreased or even "disappeared." This situation offered a favorable opportunity to evaluate the results of a house spraying campaign in terms of sandfly control, and perhaps also of disease control.

Arrangements were made for this study under the immediate auspices of World Health Organization-Interim Commission. The writer was in Greece from 6 June to 24 October 1948, with headquarters at the Athens office of the WHO-IC Mission in Greece. Laboratory facilities were generously provided by the Athens School of Hygiene.³ The general plan was to make as many observations as

¹ This work was done under a contract between the Army Medical Research and Development Board, Office of the Surgeon General, and Gorgas Memorial Laboratory, and under the immediate auspices of the World Health Organization-Interim Commission.

² Gorgas Memorial Laboratory, Panama, R. de P.

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possible on the mainland and to devote special attention to the District of Canea in Crete with its well known foci of leishmaniasis.

THE SANDFLIES OF GREECE

There are listed below the eleven species of this subfamily which have been previously recorded from the Greek mainland and from Crete (7-15). The nomenclature of Theodor's (16) recent revision of Old-World *Phlebotominae* has been followed.

Phlebotomus (Phlebotomus) papatasi Scop. 1786.

Phlebotomus (Larroussius) major Ann. 1910.

" " *perfiliewi* Parrot 1930. (Macedonia only.)

" " *tobbi* Adl. & Theo. 1930. (Not reported from Crete.)

Phlebotomus (Adlerius) chinensis var. *simici* Nitz. 1931.

" " *mascittii*⁴ Grassi 1908 (syn. *larroussiei* Lang. & Nitz. 1931). (Crete only.)

Phlebotomus (Paraphlebotomus) sergenti Parrot 1917.

" " *alexandri* Sinton 1928. (Not reported from Crete.)

Sergentomyia (Sergentomyia) minuta Rond. 1843 (syn. *P. parroti* var. *italicus* Adl. & Theo. 1931).

Sergentomyia (Sergentomyia) theodori Parrot 1942 (syn. *P. minutus* Adl. & Theo. 1926).

Sergentomyia (Sergentomyia) bruchoni Parrot 1935. (Island of Poros only.)

The application of residual DDT was begun in 1945 and got under way in earnest in 1946, when there were sprayed 700,000 houses and outbuildings, mostly in villages (5). It was estimated that 80 per cent of the population in malarious areas throughout continental Greece and the islands had been "protected," a proportion which has since been increased. The inner walls and ceilings of houses,

Dr. J. M. Vine, Chief of WHO-IC Mission in Greece, Colonel D. E. Wright and Mr. Paul Bierstein, Sanitary Engineers, and others of the Athens staff;

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Professor John Hadjinicolaou, Dr. G. Belios and Dr. P. Issaris, members of the School of Hygiene staff, whose personal kindnesses were continuous and innumerable. We wish to thank especially Professor Hadjinicolaou, who with notable generosity and patience shared his laboratory with us and who, together with his assistant, Mr. I. Petritis, took an active part in the field work.

We had the good fortune to secure the services, as interpreter and assistant, of Mr. Marios Balodimos, a sixth-year medical student, who quickly became an invaluable aid in both the field and laboratory phases of the work.

⁴ The status of *P. mascittii* has been in doubt. It was considered by Adler and Theodor (7) as probably an aberrant form of *pernicius*, while Saccà (17) and Parrot (18) have held that *larroussiei* was probably identical with *mascittii*. That *mascittii* is a valid species is supported by the writer's examination of the only known eotype of Grassi's material found among Newstead's *Phlebotomus* slides in the British Museum. The results of the study of this specimen were forwarded to Dr. Saccà, but at his request will be presented in a separate note.

stables and other outbuildings have been treated at the rate of 2.0 grams of DDT per square meter. The solutions used at first, usually in kerosene, were largely replaced in 1947 and 1948 by water emulsions. Cylindrical, three- or four-gallon, hand-pressure sprayers have been standard equipment. Some swampy areas have been sprayed from the air, which is the only current antilarval measure.

The work of various investigators of sandfly-borne diseases has shown that sandflies, as in many regions about the Mediterranean, are normally abundant throughout most of Greece and Crete (7-15,⁵). Identified collections have totaled thousands of specimens and have furnished data as to the distribution and relative abundance of the different species. There were no precise data, however, regarding the abundance of sandflies immediately preceding the spraying. It was necessary, therefore, to make a rough reconstruction of the situation on the basis of reports by local residents. It has been our experience in various countries that where sandflies are abundant most of the people recognize *Phlebotomus* with considerable accuracy and distinguish them from mosquitoes. Greece proved to be no exception. Some know sandflies by sight. (The dominant Mediterranean sandfly, *P. papatasi*, may rest in great numbers in plain sight on smooth, light-colored walls.) More commonly the people distinguish them by their silent flight, nocturnal habits and to a certain extent by the peculiarly irritating nature of the bite. In the various places where we worked a substantial majority of the people gave reports of sandflies formerly present in annoying abundance and usually added bits of corroborating circumstantial evidence. They also, almost without exception, volunteered the information that sandfly annoyance had ceased with the first DDT treatment. In regard to the latter point, somewhat more than ordinary weight may be given to popular reports since there is now a tendency to question the efficacy of DDT (or the integrity of those applying the material). Having experienced an undreamed-of absence of houseflies following the first treatment, the faith of the people was somewhat shaken by the re-appearance of the now very abundant DDT-resistant flies (combined with the change from the earlier kerosene solution to the emulsion or wettable powder, thought by the people to be "adulterated" with water obtained locally).

An index of normal sandfly abundance during the season of 1948 we made an effort to locate villages or groups of houses where DDT had never been used, or at least not recently. We met with success, as shown below, in certain villages near Athens, as well as in one of its suburbs. The very numerous sandflies found in some cases we took to indicate that 1948 was not an off-year for *Phlebotomus*.

We soon found that household sprays were in very common use, particularly in urban centers, and represented a factor very difficult to measure or estimate. For example, of about 70 households sampled in Athens, about 85 per cent used such sprays at least occasionally. Most of these sprays contained DDT. The crystals could be seen on the outside of the sprayer or bottle. Casual observations in other countries had shown that *Phlebotomus* was usually scarce in houses where

⁵ Professor Hadjinicolaou made rather extensive collections of sandflies in 1938-1939 in the Athens area. Our data as to abundance and distribution of species in unsprayed areas are comparable with his.

sprays with either pyrethrum or DDT had been used the previous night, and that this method provided good temporary protection from sandfly bites. In Athens the cost and relative scarcity of sprays made their regular, daily use uncommon. A scattering of sandflies and occasionally as many as forty could be found in houses which used sprays "sometimes." The principal target was houseflies and the spraying was done largely during the day, although in a few cases sandflies were primary objectives. In one case with before-and-after observations by us, regular space-spraying had reduced the sandflies from 200-300 to a dozen or so.

Technique. Searches for sandflies were made with the aid of tobacco smoke and flashlights. Smoke was used even on smooth white walls, since if sandflies are scarce their enforced movement will reveal them at once. Furthermore they are easier to catch after having reacted to the smoke. Specimens were caught in a suction tube and transferred immediately to 70-per cent alcohol.

No insect repellent was used at any time, although we had constantly available the Army repellent. In general, the sandflies were either very scarce or were not attacking persons actively moving about in the open. Even in the heavy flights of *perfiliewi* encountered in Italy, the exposure was relatively brief and it was decided that the slight disease hazard was outweighed by the desirability of not interfering with sandfly activity.

Oiled paper traps. Sandfly traps consisting of sheets of paper smeared with castor oil were used successfully by Kirk and Lewis (19). Late in the season we gave the method a trial. A comparative test was first made with sheets of white bond paper, typewriter size (21.5 x 28 cm.), and pieces of ordinary window screening. The latter would hold a tiny drop of oil at each intersection of the wires, and it was thought that it would be easily manipulated in the field. On the first trial, however, with paired paper and screening, the only sandflies caught were on the paper, and the screening was abandoned. A final, and apparently satisfactory, arrangement was to tack the paper onto wooden frames (Fig. 6). These were made of thin strips, 2.5 x 26.5 cm., nailed with their flat surfaces together to form a square. The paper was fastened with thumb-tacks in the mid-plane of the frames so that they could be stacked without the papers coming in contact with one another. The oil was applied with a brush before leaving the laboratory. The frames could be hung on walls like pictures or placed on articles of furniture without undue messiness or apprehension on the part of the householders. The frames were picked up the following morning and examined at leisure in the laboratory. The specimens, which were in good condition and at times still alive, could be picked off by "lifting" a small quantity of the surrounding oil with fine curved forceps, or by putting a needle under the specimen. By transferring directly to phenol the oil was got rid of and the specimens were cleared for identification. In the field they could be transferred to alcohol. One application of oil would last several days. If the paper became fouled with debris or too many other insects, it could be wiped clean with xylene and used repeatedly.

Permanent stained preparations were made by the phenol-copal method (20), the final mount being in copal. Most of the catches, however, were identified

after clearing in pure phenol (with just enough water to keep it liquid) after which they could be returned to the alcohol. This method is rapid, does not injure the specimen and permits a high degree of accuracy once the species of a region are known. All the characters of taxonomic importance with the exception of the cibarium can be seen. The posterior part of the pharynx with its armature is rarely obscured by the eyes. We have found it convenient to arrange 6-12 specimens around the edge of the concavity of a hollow-ground slide, where they will be in contact with the cover-glass but not crushed when the liquid evaporates, and where they will be out of the way of the central bubble. A simple sketch diagram showing the orientation of the specimens in the circle, on which may be noted the identifications as they are made, makes it easy to be sure that all the specimens have been checked and, furthermore, makes it convenient to refer later to any of special interest. Such temporary preparations may be left indefinitely, adding a little phenol from time to time. Camera lucida drawings or photographs may be made with little danger that the specimen will move during the process. It is often the case that the spermathecae and their ducts, free of distortion, may be more clearly seen in such phenol preparations than at any subsequent stage of mounting. Advantage may be taken of this fact when new species or aberrant forms are encountered.

It may be remarked that after alcohol storage for a number of months, treatment with caustic may not clear up the tissue around the spermathecae. It is therefore desirable, although not practiced on this occasion, to avoid alcohol altogether with at least part of any given collection and store it as dried specimens.

OBSERVATIONS

Athens and suburbs. DDT history: The extensive metropolitan area of Athens, including Piraeus and the suburbs stretching along the sea, had never been included in the regular house spraying program, since the area was not malarious. However, a considerable amount of residual spraying had been done within this area from 1945 on. All military installations of the British, Greek and American forces had been treated. They included buildings in the center of Athens as well as in the outskirts. In addition a considerable number of both public and private buildings had been sprayed. It was apparently a common occurrence for those with official or personal contacts to have their houses sprayed. Among 70 private residences sampled at random, four were found to have had the residual spray in 1946, 1947 or 1948, and two of these houses had been sprayed twice. Colonel D. E. Wright informed us that about one-fourth of the personnel of UNRRA had sandfly fever in 1945. In 1946 their quarters were sprayed and no cases at all were reported among some 2000 employees of this organization.

During the summer of 1946 a large portion of the suburb of Nea Smyrni, between Athens and the sea, had been sprayed with DDT from the air. Residents of this quarter maintained that the sandflies, which had been very abundant, ceased to trouble them for the rest of that season and, indeed, had not been

numerous in the following season. In the fall of 1947 practically all of Athens was sprayed from the air as a fly-control measure in connection with the Egyptian cholera epidemic. Since this was late in the season its effect on *Phlebotomus*, if any, would not be obvious.

Athens; house survey: A survey of houses in several districts of Athens was carried out, chiefly by Mr. Balodimos, at odd times during July and August. There were recorded on printed forms the names, addresses, number of persons, domestic animals, type of building and walls, history with regard to *Phlebotomus*, the use of household sprays, treatment with residual DDT. A search was also made for *Phlebotomus*. The districts were Ambelokipi and Gizi-Polygonon, in the northeast corner of Athens, and Pyritidopirion-Agios Sabas in the western part. In the two former districts 63 houses, with 292 persons, averaged a little over four sandflies per house, with 32 negative and three houses accounting for two-thirds of the total collections (91 ♂, 190 ♀ *papatasi*). In the third district the incidence was greater, with eight houses averaging 17 sandflies (54 ♂, 82 ♀ *papatasi*). In this survey we learned of the widespread, albeit irregular and inefficient, use of household sprays. There was a general awareness of DDT on the part of the people and a number of them stated that the formerly numerous sandflies had decreased with the advent of DDT, even though their particular house had not been treated, and gave the year 1945 or 1946 as the beginning of this decrease.

A few groups of houses in the center of Athens were visited in the evening. An apartment building, the residence of a colleague, had had very few sandflies the past two years compared with formerly. There had been no special treatment with the exception of household sprays. Living quarters, corridors and courts were negative, as were also the walls surrounding a garden. A few blocks away a small house, one of several around an open court-yard, yielded 1 ♀ *papatasi*, with none on the outer walls. During June and July an occasional specimen of *papatasi* was taken biting in a fifth-floor room of a hotel in the center, and in September, in a residence just south of the Acropolis.

Athens suburbs; Nea Smyrni: This suburb between Athens and the sea, built up since 1922, consists of small separate houses, most of them with gardens. A friend, Mr. Harry A. Stephopoulos, of the Ministry of Health, who has been most helpful and to whom our best thanks are due, has been a resident of Nea Smyrni for a number of years. He informed us that sandflies were extremely annoying prior to the aerosol of 1946, but that thereafter for the rest of that season, the annoyance had ceased, and that this had been the general experience of others in that suburb. His house was treated with residual DDT in the spring of 1947, with no subsequent treatment except a household spray used in a chicken-coop, and the aerosol in the fall of 1947. His house was visited several times during the summer of 1948, both by day and in the evening. We found an occasional sandfly in the house and two or three in an unsprayed half-basement, all *papatasi*. No sandflies were seen outside nor were bites felt, although formerly there had been much annoyance when sitting outside in the evening. Three other houses a number of blocks distant, without previous residual spray, were negative in

daytime searches. A small dairy on the edge of this suburb had at least 50 sandflies in the house. Those caught consisted of 5 ♂ 5 ♀ *papatasi*. In the stable with horses and cattle, two were seen on one occasion, while a storage building was negative. The extreme scarcity of sandflies in stables, pigpens and chicken-coops turned out to be a feature of all our observations throughout the season. This was in contrast to the experience of Caminopetros (8) whose catches throughout the summer of 1933 in stables on the outskirts of Athens averaged about 46 *papatasi* plus a scattering of *major*, *tobbi* and *sergenti*, with a slightly higher rate in kennels. These catches were consistently much greater than his house collections. We have no explanation for the differences between his results and ours.

Elliniko: This suburb lying along the sea beyond the Hassani Airport is similar to Nea Smyrni in that the houses are separated by open ground and gardens. It has long been notorious for its sandflies. Mr. Stephopoulos informed us that the British had had numerous troops living in this area and in 1945 suffered severely from sandfly fever until DDT was applied. He accompanied the British Army authorities at the time the spraying was first done in buildings occupied by their men, and was our guide on our first visit. Many of the houses were badly damaged during the war and were still vacant. On 14 June one house had an estimated 200-300 sandflies, of which a number were caught (22 ♂ 27 ♀ *papatasi*). A pigpen and chicken-coop were negative. The DDT history of the house was unknown. The people were new tenants and had not yet equipped themselves to cope with the extreme sandfly annoyance. On 5 July, however, the place had been more or less regularly treated with a spray containing DDT and only about a dozen sandflies were to be found. Four or five other occupied houses, most of them with household sprays in use, yielded a total of 9 ♂ 5 ♀ *papatasi*. The damaged vacant houses were consistently negative, even in those rooms still intact. Pigpens and chicken-coops were consistently negative.

A colleague living in a large residential suburb just north of Athens had formerly had severe sandfly annoyance, both indoors and out. The house was treated with residual DDT in the spring of 1947. Thereafter, throughout the seasons of 1947 and 1948, with limited use of household sprays, there had been no further difficulty. On two evening visits (13 June, 3 September) no sandflies were found. Colleagues, associates and others were queried throughout the summer for information about neighbors and friends who might be troubled with sandflies, without discovering any noteworthy foci.

Rock crevices and a limestone grotto at the base of the Acropolis above the Amphitheater of Dionysus were explored on several occasions. A single *Phlebotomus* was seen in a crevice, but not caught. The distance to the nearest house was about 150 meters.

It will thus be seen that sandflies in Athens and its suburbs were at a rather low level, with many individual accounts of great annoyance two or three years previously. That the sandfly incidence was potentially much higher in 1948 was shown by the numerous sandflies in several places, both in the city and in its suburbs, where no recent measures had been taken. Actually, if there are considered only those houses where we found any sandflies at all, our average

house catches in Athens proper (10.3 *papatasi*) nearly equalled those of Caminopetros (8) in 1931-1933 (11.6 *papatasi* plus a scattering of other species which we did not find at all). Our maximum house catch (one hundred twenty) was greater than his (73 *papatasi*). In assessing the current situation in Athens it is impossible to estimate the roles which various factors may have played,—the peripheral effect of the many buildings treated with residual DDT, the use of household sprays, and the treatment from the air. The only species in our Athens collections was *papatasi*.

Villages in Attica: The great majority of the villages in Attica had been sprayed in 1946, 1947 and 1948. A number were visited and, as shown in Table 1, were found to have very few sandflies, but we obtained consistent reports of much annoyance which stopped with the first spraying. It was fortunate for our purposes, however, that certain non-malarious places had been left unsprayed. Observations in the latter at various times throughout the summer we have used as an index of normal sandfly incidence in 1948.

Some of the village surveys were made by Mr. Balodimos, at times in the company of Professor Hadjinicolaou and Mr. Petritis, the members of the party being indicated by their initials. Data were recorded on the same printed forms used in the Athens survey. Village houses are small, usually of one or two rooms, plastered and whitewashed inside and out. The ceilings are usually finished. The cooperation of the people, who were very kind and hospitable, was excellent. Not only was admittance never refused, but we were usually cordially invited to enter and were aided whenever furniture had to be moved or ladders brought to reach the higher ceilings. Household sprays are rather less commonly used in villages than in Athens. Identified collections are summarized in the Table.

Sprayed villages: In sampling sprayed areas special effort was made to find any houses which had not been sprayed or which had been missed in the 1948 treatment.

The villages of *Liosati*, *Afidne* and *Kapandriti*, a group about 30 kilometers northeast of Athens, were sampled 26 August (MB). All had been thoroughly sprayed. Seven houses were negative, with practically no household sprays used.

Kato Souli, a village east of Marathon, had been very malarious and had been treated for four consecutive seasons. On 27 August (MB) six houses were negative, with reports in most cases that sandflies had been abundant before the first treatment and in one case that there were still a few.

Nea Penteli is a wind-swept locality on Mt. Penteli. Five houses were negative on 22 July (MB), two of them sprayed in 1947, the others untreated. Only two of the five reported moderate sandfly annoyance before the 1947 treatment. On 30 August (MB) three other houses which had been sprayed in 1948 were negative, two houses reporting a few sandflies previous to the treatment. As in certain other windy places, sandflies were apparently not normally abundant.

On the *Sounion Peninsula*, extending 45 kilometers southeast of Athens, most of the towns and villages had been sprayed. A few places left untreated are mentioned below.

In the town of *Lavrion*, 17 July (MH, JH, MB, IP), we learned of one un-

TABLE 1

Identified collections of Phlebotomus in Greece

Shows principally the distribution of species at different times and places. Relative abundance is indicated roughly by the figures following each location, which represent the total number of visits either to the same or different houses. Totals do not include some instances where sandflies were extremely abundant and were merely estimated, but where actual collections consisted almost entirely of *papatasi*.

Negative observations, which were the rule in sprayed houses and were common in sprayed village areas, are included only where specifically noted.

Sprayed and unsprayed refer to residual DDT.

DATE 1948	PLACE	NUMBER OF HOUSES OR VISITS	<i>Phlebotomus papatasi</i>		<i>P. chitensis</i>		<i>P. major</i>		<i>P. tobbi</i>		<i>P. sergenti</i>		<i>P. alexandri</i>		<i>Sergentomyia minuta</i>	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Jul.-Aug.	<i>Athens</i> , unsprayed houses															
	Ambelokipi.....(18 neg.)	36	30	79												
	Gizi-Polygonon.....(12 neg.)	23	61	111												
	Pyritodipirion.....(1 neg.)	8	54	82												
	Other districts.....	3		5												
Jun.-Jul.	<i>Athens suburbs</i> , unsprayed															
	Nea Smyrni.....(2 neg.)	5	5	7												
14 Jun. 5 Jul.	Elliniko.....	ca. 9	*31	*32												
	(*200-300 <i>papatasi</i> estimated in 1 house)															
Total, house catches.....			181	316												
	<i>Villages in Attica</i>															
	<i>Sounion Peninsula</i>															
17, 23 Jul. 21 Aug.	Unsprayed houses in 4 villages.....	8	8	14	3	4	1		1						1	
	Unsprayed areas; isolated houses..	6	1	8	1	2	1									
21 Aug.	Daskalia, unsprayed.....(1 neg.)	5	8	5	1										2	
4, 18 Jul.	Loutsa, unsprayed.....	ca. 5	8	5	2	49									1	
20 Aug.	Ekali, unsprayed.....	1					3								5	4
	<i>Melissia</i>															
	<i>Unsprayed section</i>															
Jul.-Oct.	House No. 11, inside.....	9	*6	*6	2	3	1	3				13	41			
	(*100-150 estimated at first visit)															
	Outside.....	11 evenings	3	1	2	6	4	8				109	86			
8 Sep., 4, 8, Oct.	Oil-paper traps.....	3 nights	8	4		1	3	7				3	2		1	
Jul.-Oct.	Other houses, inside.....	12	124	164	2	3	3				1	1	7	6	3	1
	Outside.....	5 evenings										1	1			
	<i>Sprayed section</i>															
27 Jun.	Before 1948 spray.....	5	1	1	5	4										
5 Aug.	Same 5 houses after spray															
	Inside, neg.															
	Outside.....	1 evening	1				10	3				6				
8 Jul.	Sprayed house, inside.....	1	1	1												
	<i>Kinetta, unsprayed</i>															
25 Jul., 7, 20 Aug.	Houses, inside.....	16	525	445	22	4	4	1	7	5	2	23	12	123	59	
19 Aug.	5 houses, outside.....	1 evening	25	8	1				2	1	4	1		1	9	
Totals: House catches.....			682	649	35	69	12	8	7	6	1	3	43	59	135	64
Outside, night.....			37	13	3	7	45	18	2	1	4	1	119	90	9	1
Total, Attica villages.....			719	662	38	76	60	26	9	7	5	4	162	149	144	65

TABLE 1—(Continued).

DATE 1948	PLACE	NUMBER OF HOUSES OR VISITS	<i>Phlebotomus papatasi</i>		<i>P. chinensis</i>		<i>P. major</i>		<i>P. tobbi</i>		<i>P. sergenti</i>		<i>P. alexandri</i>		<i>Sergentomyia minuta</i>	
			♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
	<i>Crete</i>															
	<i>City of Canea, unsprayed houses</i>															
	<i>Agios Ioannis</i>															
23 Jul., 13-27 Sep.	Houses, inside..... (24 neg.)	over 40	15	30	1						4	7			1	1
	(12 sprayed, also neg.)															
	Outside.....	9 evenings	1	1			4	4			1					
18 Sep.	Oil-paper traps, inside.....	1 night	6	2			1				1					
18 Sep.	Oil-paper traps, outside.....	1 night													2	2
	<i>Splanzia</i>															
26 Jul., 4 Oct.	Houses, inside..... (13 neg.)	17	1	4							1	1				
4, 5 Oct.	Outside.....	2 evenings									1	1				
	<i>Halepa</i>															
28 Sep.	Houses, inside..... (6 neg.)	9		2	1											
	<i>Kounkapi</i>															
1 Oct.	Houses, inside..... (11 neg.)	12		1												
	<i>District of Canea, rural areas</i>															
24 Jul., Sep.	Isolated unsprayed (?) bldgs.....	3		1	1	2						2				
20 Sep.	Unsprayed wall, outside.....	1 evening				1										
	<i>Charakies, sprayed village</i>															
17 Sep., 7 Oct.	Farmyards, outside,															
	Oil-paper traps.....	2 nights			1	1	1									
6, 10 Oct.	Several caves, inside.....	2					18	8								
	Oil-paper traps.....	1 night					1									
Totals: House catches.....			22	40	1	4	1				6	10			1	1
Outside, night.....			1	1	1	2	5	4			1	2			2	2
Cave catches.....							19	8								
Total, Canea.....			23	41	2	6	25	12			7	12			3	3
Grand total, 2756 sandflies.....			922	1018	40	82	85	38	9	8	12	16	162	149	147	68

treated house. It was negative and we were informed that there had never been sandflies. However, three nearby houses reported former abundance, in one case extreme, which had ceased with the first spraying in 1946. We saw one sandfly in a sprayed living room, one of the rare instances of this phenomenon.

In *Keratea* and *Sounion*, 23 July (MB), seven houses, all but one or two sprayed in 1947, but not in 1948, yielded 1 ♀ *papatasi*, 1 ♂ *minuta*. Five of the seven gave histories of former sandfly annoyance, with no valid information in the other two.

The *Sounion* Peninsula was visited for the third time on 21 August (JH, MB, IP) principally to seek out houses in sprayed villages which had missed the treatment. Four such houses in *Liopessi* reported sandflies as formerly abundant and still present to a certain extent. Two of the houses, with almost daily use of household sprays, yielded five sandflies, while in the other two with infrequent spraying, 16 were caught. In *Koropi* four untreated houses with little or no household spraying harbored an estimated total of about 20 sandflies. This village had been sprayed from the air in 1946 with an immediate decrease of sandflies reported. Three houses in *Markopoulon* were negative, two sprayed in 1948, one last treated in 1946. In *Keratea* two houses sprayed in 1948 were negative

while three sandflies were caught in one last sprayed in 1947. Total catch in these four villages: 8♂ 13 ♀ *papatasi*, 3 ♂ 4 ♀ *chinensis*, 1 ♀ *major*, 1 ♀ *tobbi*, 1 ♂ *minuta*.

The data for the sprayed portion of the village of *Melissia*, where special observations were made, are reported below.

Unsprayed areas. Sounion Peninsula: Along the six-kilometer stretch of road at the tip of the peninsula between Lavrion and Sounion are a number of scattered houses, mostly summer residences, which had not been sprayed. Eight houses were visited 17 July (MH, JH, MB, IP). In only one house were sandflies even moderately abundant (20–30 seen, identified sample all *papatasi*), with a few seen or caught in three others. Sandfly annoyance was reported by all four. A new, unoccupied house was negative. Two other places were mere shacks with open fires and numerous openings at the eaves. The remaining house, of a type which could have furnished suitable sandfly shelter, reported no sandfly annoyance. Near the temple ruins at the extreme end of the peninsula, gun emplacements and dugouts were negative. This area is wind-swept and sandfly abundance is apparently not great. Identified catch: 1 ♂ 7 ♀ *papatasi*.

Daskalio, a small village on the coast east of Keratea, had never had residual DDT. On 21 August (JH, MB, IP) five houses reported "some" to "many" sandflies. One house was negative while in the other four were caught 16 sandflies, including two in a stable with goats (8 ♂ 5 ♀ *papatasi*, 1 ♀ *chinensis*, 2 ♂ *minuta*).

South of *Markopoulon* two isolated unsprayed stone buildings were found (MH, JH, MB, IP). One, used at times as a stable, was negative. The other, a house apparently not regularly occupied, contained 1 ♀ *papatasi*, 1 ♂ 2 ♀ *chinensis*, 1 ♂ *major*.

Ekali is a village-suburb 17 kilometers north of Athens on the Kifissia road. A colleague collected in his unsprayed room on 20 August, 3 ♂ *major*, 5 ♂ 4 ♀ *minuta*.

Loutsas, a small village on the coast about 24 kilometers east of Athens, largely composed of scattered summer residences, had never been sprayed. On 4 July Dr. Belios caught during the daytime, mostly in one house, 1 ♂ *papatasi*, 2 ♂ 48 ♀ *chinensis*. The preponderance of *chinensis* was wholly exceptional in our experience. Two weeks later Mr. Balodimos in several other houses caught 7 ♂ 5 ♀ *papatasi*, 1 ♀ *chinensis*.

Melissia is a village 12 kilometers northeast of Athens at the foot of Mt. Penteli (Fig. 1). It covers a large area with most of the houses 50 to 100 meters or more apart. The residents include a number of transients, summer residents and a number of permanent residents who are at least part-time farmers. Most of the village area is cultivated with vineyards and gardens. There are a few cattle, sheep and goats.

The regular treatment with residual DDT (22–27 June) was interrupted, leaving a group of about a dozen houses on the northeast edge of the village unsprayed. At our request the work in this non-malarious place was not resumed, so that observations could be made throughout the season.

Sprayed sections of Melissia: On 27 June, with Dr. Belios, five houses were

searched just before being sprayed. Four of them, treated in 1947, yielded a total of 1 ♂ 1 ♀ *papatasi*, 5 ♂ 4 ♀ *chinensis*. The fifth, which had never been sprayed, was a small farm with a cow, donkey and chickens. The house contained a fair number of sandflies with one or two found in the stable (13 ♂ 8 ♀ *papatasi*, 1 ♂ *chinensis*, 1 ♂ *major*, 1 ♂ *alexandri*). This same group of houses at an evening search 5 August (MH, GB, MB, IP) were all negative inside. Outside were caught a total of 20 sandflies, most of them at the small farm. At the latter, 3 ♂, 1 freshly fed ♀ *major* were taken on the outside of the stable near a tethered donkey. No sandflies alighted on the animal during several minutes of observation, nor were any of our party bitten. Total evening catch: 1 ♂ *papatasi*, 10 ♂ 3 ♀ *major*, 6 ♂ *alexandri*.

In another part of the village on 8 July (MH, JH, MB, IP) nine sprayed houses were negative with the exception of one, in which 1 ♂ *papatasi* was caught in an unsprayed closed cupboard and 1 ♀ on the bedroom wall (one of the two instances during the summer when sandflies were found in a sprayed house). In another house a room built since the spraying (22 June) contained 2 ♂ 7 ♀ *papatasi*, while the sprayed part was negative.

On the evening of 7 August (MH, JH, MB, IP) a tour was made of a number of places in the sprayed area, most of which had not been previously visited. No sandflies were seen on the outer walls of houses, and the people reported no present annoyance, whereas there had been formerly. At an outdoor cafe with bright lights hung among the trees, we were informed that there were now no sandflies. One sandfly was seen on the wall of the cafe building. Several long low apartment buildings along the road near the village center reported no sandflies. None could be found on the outer walls nor in two or three unsprayed bedrooms.

Unsprayed section of Melissia: Immediately beyond the group of sprayed, negative houses visited 8 July, was the untreated area. Four houses were searched. In one, where household sprays were used, only two sandflies were seen, while in the other three they were very numerous, with estimates ranging from 100-150 (House 11) to 300-500 (House 10). In the latter, one corner near the ceiling was estimated to have 50-100 sandflies. Those caught were: 68 ♂ 75 ♀ *papatasi*, 1 ♀ *chinensis*, 1 ♂ *sergenti*, 2 ♂ *alexandri*, 2 ♂ *minuta*.

An evening visit 13 July (MH, JH, MB, IP) showed a striking difference between Houses 10 and 11, which were therefore chosen for subsequent observation during the season. Neither one had been sprayed in 1947 or 1948. In both there were many *papatasi* inside. About 50 *alexandri* were caught on the outer walls of H-11 during the evening, with only two seen outside H-10. These two houses were about 50 meters apart, H-10 set out in the open with no outbuildings, trees or high vegetation, while H-11 was on sloping ground with a succession of low, attached sheep-sheds on the uphill side, several trees and stone retaining and boundary walls. The latter house was definitely better protected from the wind, of which there seemed to be an unusual amount during the summer.

Beneath a cement porch at H-10 there was a shallow cellar with walls of loose stone, occupied by a man, a goat and stray chickens. There was also a lean-to chicken house with stone and adobe walls. In spite of the great numbers of sand-

flies in the house, these two outer structures yielded very few sandflies either by day or by night. Likewise the sheep-sheds and long stretches of loose stone retaining walls of H-11 yielded practically no sandflies. Our attention was therefore directed chiefly to the houses themselves. By early August the sandflies (chiefly *papatasi*) inside the two houses as well as in others nearby had decreased to a few stragglers. This may have been due in part to the very occasional use of household sprays instituted since our first visits, but it seems inadequate to account for the general decrease of *papatasi* which was still very abundant in another village, Kinetta, the latter part of August. Since these houses were within 100 to 200 meters of the sprayed section of Melissia we may have been encountering a peripheral effect of residual DDT.

On 5 August (MH, GB, MB, IP), one of the few very still evenings we happened to have, there was an extraordinary flight of *alexandri* at H-11. Between 9:45 and 10:15 we caught over a hundred of this species and a scattering of three others and could have taken many more. They were on the outer walls of the house, but especially on the ceiling of one room near an open window, on the sill of which a lamp had been set. The people, who had been sitting outside all evening, said they were being bitten somewhat, while our party of four, actively moving about, felt no bites. H-10 unfortunately was not visited. Total evening catch: 2 ♂ 1 ♀ *papatasi*, 1 ♂ *chinensis*, 2 ♂ 1 ♀ *major*, 53 ♂ 60 ♀ *alexandri*.

No comparable flight of *alexandri* was seen again, even with the same arrangement of lights, and catches of this species declined after the end of August.

On 8 September (MH, JH, MB, IP) at H-11 a trial was made of the castor oil-paper traps. Several sheets caught a total of 8 sandflies during the night. There had been heavy rain early in the evening with considerable wind thereafter. On 20 September (JH, IP) about 30 sandflies were caught outside H-11, with *chinensis* and *major* forming a larger proportion of the catch than previously (6 ♀ *chinensis*, 1 ♂ 6 ♀ *major*, 5 ♂ 11 ♀ *alexandri*). During September H-10 never had more than five or six sandflies inside, with none seen outside. On 4 October (MH, JH, IP) the frames with castor oil-paper were set around the house and outbuildings at H-11. The evening was clear, still and cool. Only one or two sandflies were seen. During the night, of a total of eleven frames, two placed in the privy and one hung on a stone boundary wall caught 28 of the total of 33 sandflies, with *major* comprising all but five specimens of the total catch (1 ♂ 1 ♀ *papatasi*, 1 ♀ *chinensis*, 26 ♂ 2 ♀ *major*, 1 ♂ *alexandri*, 1 ♀ *minuta*). At the last visit to Melissia, 8 October (JH, IP) the frames were set out in approximately the same positions. *P. major* made up two-thirds of a somewhat reduced catch (5 ♂ 1 ♀ *papatasi*, 7 ♂ 5 ♀ *major*, 1 ♂ *alexandri*).

It will be noted that the preponderance of *major* late in the season was revealed only by the use of the oiled paper traps. Most of them were caught 10-20 meters from the house. We found no point in the stone walls here or elsewhere at which this or any other species was emerging. It is clear that, along with the search for and study of outdoor resting and breeding places of Phlebotomus in general, the biology of *major*, the supposed vector of kala azar, is in need of thorough study.

Kinetta, a village consisting mostly of scattered summer residences along the

sea 55 kilometers west of Athens, was not malarious and had never been sprayed. On 25 July Dr. Belios collected about 100 sandflies in one or two houses which included specimens of *tobbi*, which we had not found in catches identified up to that time. On 7 August (MH, JH, GB, MB, IP) three summer residences along the road surrounded by nearly bare, sandy soil, had a great many sandflies, the catches averaging 171 per house (85 per cent *papatasi*, 11 per cent *minuta*). Sandflies were moderately abundant in the hotel and very scarce in a house with regular use of a spray containing DDT. In an open, unfinished house were caught about 30 sandflies, nearly all *minuta*. Two small farms 200 meters from the main road harbored only about one-fourth as many sandflies as the summer residences, with relatively fewer *papatasi* and more *minuta*. At one of the farms sandflies were nearly as numerous in the stable as in the house (24 and 33 respectively), an exception in our summer's observations. Those in the stable were nearly all males, in the house nearly all females. Total daytime catch in nine buildings: 287 ♂ 202 ♀ *papatasi*; 9 ♂ 1 ♀ *chinensis*, 2 ♂ 3 ♀ *tobbi*, 1 ♀ *sergenti*, 9 ♂ 7 ♀ *alexandri*, 73 ♂ 28 ♀ *minuta*.

Evening observations were made 19 August (JH, MB, IP) from 6:30 to 10:30. At 6:30 *papatasi* was still abundant in the summer residences, though less so than on the morning of the previous visit. By 9:00 or 9:30 the sandflies inside houses had dropped by about two-thirds. While the evening had been fairly still it became very still at 9:30 and sandflies found on the outer walls increased somewhat, but had fallen again by 10:15. Of the 52 specimens caught outside, the proportions of *papatasi* and *minuta* were 63 and 17 per cent respectively, with species of the *major* and *sergenti* groups, of which there was no notable flight, comprising 19 per cent (25 ♂ 8 ♀ *papatasi*, 1 ♂ *chinensis*, 2 ♂ 1 ♀ *tobbi*, 4 ♂ 1 ♀ *sergenti*, 1 ♀ *alexandri*, 9 ♂ *minuta*). The following morning 449 sandflies were caught in six houses, *papatasi* 78 per cent, *minuta* 14 per cent, the others 8 per cent (191 ♂ 160 ♀ *papatasi*, 11 ♂ 2 ♀ *chinensis*, 1 ♂ 1 ♀ *major*, 4 ♂ 1 ♀ *tobbi*, 1 ♀ *sergenti*, 12 ♂ 1 ♀ *alexandri*, 39 ♂ 25 ♀ *minuta*).

Taking the group of villages in Attica as a whole, in those which had been treated with residual DDT sandflies were very scarce, even in those houses which had escaped the 1948 treatment but were in the midst of treated houses. Former sandfly annoyance had sharply decreased or ceased with the first spraying. Treated houses were consistently negative in daytime searches, with rare exceptions of an occasional sandfly. This virtual freedom from sandflies also applied to the village area out-of-doors where the spraying had been done in previous years as well as in 1948. In places first sprayed in 1948 a few sandflies could be found outdoors in the evening. In unsprayed places the sandfly incidence, where undisturbed by household sprays, was very high in a number of individual houses and moderate in most of the others. Sandfly incidence was apparently not very great in some wind-swept areas.

P. papatasi was by far the dominant species and was found almost entirely in occupied houses. The one catch of nearly pure *chinensis* in Loutsa was exceptional. Stables, pigpens and chicken-coops had very few sandflies of any species, even when *papatasi* was abundant in nearby houses. Stone retaining and boundary walls were likewise negative on direct examination either by day or

by night. Indeed, we were unable to determine the outdoor resting or breeding places of any species. In night observations, the only heavy flights were those of *alexandri* at one house in Melissia. We never found *papatasi* on the outer walls in numbers corresponding to the house catches, which was also the experience of Adler *et al.* in Crete (10). It is known that certain species which do not rest in houses may have flight periods between midnight and dawn (*e.g.*, *wenyoni* (21)) and if any such species were present it could have been missed. The variety of *chinensis* in the Middle East is found principally after midnight (21). However, the sandflies recorded for Greece are known to be abroad in the evening. *Major*, *chinensis*, *tobbi* and *sergenti* were slightly more numerous in evening catches than in houses during the day, but were seldom represented by more than a scattering of specimens, with the possible exception of the October night catches of *major* at Melissia.

CRETE

Adler *et al.* (10) made a careful study of *Phlebotomus* and leishmaniasis in Canea, Crete, from July to November 1934. They paid particular attention to the sharply limited foci of leishmaniasis within the town itself,—kala azar, chiefly in the section known as Agios Ioannis (Figs. 4, 5) and oriental sore in the old Turkish quarter, Splanzia (Fig. 3). (See map of Canea with leishmaniasis foci (10, 22).) *P. papatasi* was abundant in both and formed the large majority of house catches. In evening collections out-of-doors in Agios Ioannis, *sergenti* was the commonest species the first half of July but later *major* predominated, with *chinensis* var. *simici* also present. In Splanzia *sergenti* was common with *major* extremely scarce and *chinensis* not taken at all. The abundance of *major* is indicated by their table showing the number of females which could be caught during several hours in the evening, which ranged from 40–50 per evening in July and August through a low of 20 the first half of September to the peak of 80–120 the first half of October, and declined to 20–30 the first half of November. Specific numbers or proportions of the other species in their total of 7000 female sandflies were not given. From their observations and experimental work they concluded that *major* and *sergenti* were the respective local vectors of kala azar and oriental sore. Mayer and Malamos (11) the following year also found *Phlebotomus* abundant. Hundreds of sandflies could often be found in living quarters. Their identified totals were: Agios Ioannis, 79 *papatasi*, 84 *major*, 9 *sergenti*; Splanzia, 88 *papatasi*, 99 *sergenti*.

Crete was visited with Professor Hadjinicolaou 21–31 July 1948, and again with Mr. Balodimos 12 September to 14 October. The latter period was chosen so as to include the peak of the sandfly season. Dr. E. Papantonakis, Medical Officer for the District of Canea, generously provided laboratory facilities at the Hygiene Center in Canea. He had similarly aided the previous investigators in 1934 and 1935 and was familiar at first hand with their work and the places where their observations had been made.⁶

As in the case of Athens, the town of Canea had not been included in the

⁶ We are also indebted to Dr. Dimou and Mr. Fragoudakis, of the Malaria Service, and to Mr. L. G. Allbaugh, of the Rockefeller Foundation, for transportation and other aid.

malaria spraying campaign, but a number of public and private buildings had had the residual treatment regularly since 1946. In Agios Ioannis several groups of houses had been sprayed by UNRRA in 1946. Between 20 and 25 November 1947 the whole town, along with other cities in Crete, had been sprayed from the air as an anti-housefly-cholera measure. Practically all the villages in Crete had been treated for three consecutive seasons. In a few cases the 1948 spraying was delayed until July or August on account of bandits.

Observations on Phlebotomus: 21-31 July 1948. In Agios Ioannis (Figs. 4, 5) a few sandflies were seen in five unsprayed houses with casual use of household sprays (caught: 4 ♀ *papatasi*, 1 ♂ 1 ♀ *sergenti*, 1 ♀ *minuta*). There was one convalescent case of kala azar contracted probably in 1946. Four houses sprayed in 1946 and one in 1947 were negative. The occupants gave good circumstantial accounts of great sandfly annoyance which ceased completely with the treatment and had not reappeared. On one evening visit outdoor searches around the group of houses with the kala azar case yielded 3 ♂ 2 ♀ *major*, 1 ♀ *sergenti*. The sprayed houses were completely negative except for 1 ♀ *papatasi* caught on a garden wall.

A number of houses in *Splanzia* (Fig. 3) bordering the military post overlooking the harbor had been regularly treated with residual DDT. Only a few sandflies were seen in several unsprayed houses (3 ♀ *papatasi*, 1 ♂ *sergenti*). We found no house with complaints of sandfly abundance.

Various villages were visited: *Charakies*, 3-4 kilometers southwest of Canea; *Kaseli* and *Spilia*, 35 and 23 kilometers west of Canea; *Brises* and *Georgopolis*, about 20 kilometers east of Canea; *Voukolies*, *Kandanos* and others between Canea and the town of *Paleochora* on the south side of the Island. In no occupied house did we find any sandflies, whether sprayed in 1948 or not, with the sole exceptions of 1 ♀ *papatasi* in the guard house at the old Venetian fort in *Paleochora*, and 1 ♂ 2 ♀ *chinensis* in a deserted stone house near *Kandanos*, the previous DDT history of both of which was unknown. Weep-holes in retaining walls, outdoor privies, stables, pigpens, chicken-coops, hollow olive trees, were likewise negative. One sandfly was seen in a hillside rock crevice. In *Paleochora* five houses just before being sprayed were negative. An evening search was made around houses not yet sprayed without finding any sandflies. In all these places we were given consistent accounts of previous sandfly abundance. The use of household sprays in rural areas is rather uncommon.

12 September to 14 October 1948. In spite of this period being at the normal peak of the season, sandflies were quite as scarce as they had been in July. Eight evening searches in Agios Ioannis, with favorable weather, resulted in a total outdoor catch of seven sandflies (1 ♂ *papatasi*, 1 ♂ 2 ♀ *major*, with three specimens lost and three more seen but not caught). On three evenings not a single sandfly was seen. Back of the two houses where we had caught most of our few specimens of *major*, there was a kennel with a litter of puppies and an open shelter with a tethered horse. No sandflies were seen in or near either one. Oiled paper traps were placed one night around these houses and inside bedrooms. Those outside caught 2 ♂ 2 ♀ *minuta*; those inside, 6 ♂ 2 ♀ *papatasi*, 1 ♂ *major*, 1 ♂ *sergenti*.

Daytime catches in *Agios Ioannis* in 46 houses, some of them visited several times, totaled 15 ♂ 26 ♀ *papatasi*, 1 ♀ *chinensis*, 3 ♂ 6 ♀ *sergenti*, 1 ♂ *minuta*. The greatest number of sandflies seen or estimated inside a house at any one time was 14. Twelve of the 46 houses had had the residual spray in 1946 and were negative, as were likewise 24 other houses not so treated. Two large caves which had been dug as airraid shelters had no sandflies, but various other Diptera were numerous. The DDT history was unknown although most of the houses on the same street had been sprayed in 1946.

Two other sections of Canea which had formerly been small foci of kala azar were also surveyed. Of ten houses in *Halepa*, at the eastern edge of the city, three yielded a total of 2 ♀ *papatasi*, 1 ♀ *chinensis*, 1 ♂ *sergenti*, 1 ♀ *minuta*. One of the negative houses, a police station, had been sprayed in 1948. Only 1 ♀ *papatasi* could be found in twelve houses in *Kounkapi*, the small section about 350 meters east of the old Turkish quarter. Evening searches, one in each of these two sections, were completely negative.

In *Splanzia* sandflies were found in only one of 14 houses (1 ♂ 1 ♀ *papatasi*, 1 ♀ *sergenti* caught, two more sandflies seen). One negative house had had residual DDT in 1946 and 1947. During two evenings, 1 ♂ 1 ♀ *sergenti* were caught out-of-doors, with one other sandfly seen. Most of the residents stated that sandflies had been abundant up to the introduction of DDT.

In addition to the above observations, on various occasions when in outdoor restaurants during the evening, searches were made on walls both illuminated and in shadow, but no sandflies were found. It is clear that in the town of Canea sandflies were at a rather low level.

Villages near Canea: In a farmhouse in *Charakies* a child had developed kala azar during the winter of 1947-1948. There had been no other case in the village. This house and others forming a group of six or seven, had been sprayed in 1946, 1947 and 1948. The formerly abundant sandflies had "disappeared" with the first treatment. Since this was apparently an instance where kala azar had been contracted in a sprayed house, special attention was devoted to it. A sick dog had been destroyed during the summer of 1947. The family had lived there continuously for some time and the child in question had not slept elsewhere. Furthermore, the family had always slept indoors, whereas many sleep out-of-doors on hot summer nights.

At our first visit in July the house and several stables were negative, which was also true on several visits in September and October. On 17 September, 22 frames with castor oil-paper were put around the large farm courtyard, outside doors and windows of house and stables, in privies, near chicken-coops and on the courtyard wall. Search up to an hour after dark was negative, except for one male caught on a frame at 8:15. Two more were caught on frames during the night, the total for the 22 frames being 1 ♂ 1 ♀ *chinensis*, 1 ♂ *major*. On 7 October a second trial of the frames was made, this time placing part of them around the other houses of the group. The results were completely negative. Culverts 200 meters from these houses at the edge of an olive grove were negative during the evening, and no bites were felt by a party of three.

Along the sea about 12 kilometers west of Canea is a village, *Agios Marina*,

largely dedicated to the manufacture of brick and tile. The clay is dug from exposed strata in nearby hills, leaving caves (Fig. 6). On 6 and 10 October were caught a total of 18 ♂ 8 ♀ *major* in two caves within 100 meters of the village. No kala azar had occurred within the recollection of those interviewed. The village had been sprayed in 1946 and 1947 but not in 1948. While sandflies were reported to have been numerous before the spraying there were said to be very few during the current season. There were complaints, however, that fleas had returned along with houseflies. Searches in several houses were negative for *Phlebotomus*. On 9 October the frames were set out around several houses, with seven frames placed at the entrance or inside caves. The 22 frames caught just 1 ♂ *major*. This was on a frame inside a cave, where four more ♂ *major* were caught free on the walls. This cave had yielded 12 sandflies on the first visit and a number had been seen the previous evening and left undisturbed.

Several other villages, *Daratzo* (Fig. 8), *Parigoría* (Fig. 7), *Bambokopoulo* and *Perivolía*, within about eight kilometers west and southwest of Canea, had all been sprayed for three consecutive seasons and all gave histories of sandfly abundance before the first treatment, with little or no annoyance since. The first of these villages (see below) had had a great deal of oriental sore and well over half the people showed scars. However, no case was found which had developed within the past two years. In the other villages we learned of four cases of kala azar since 1941, the most recent one having developed during the summer of 1946.

Sandflies were also very scarce in the small villages and scattered groups of summer residences and restaurants (mostly sprayed) on the high hill between Canea and Suda Bay, and in the village of *Khorafakia* on the peninsula to the north. One of several evening searches in this region netted 1 ♀ *chinensis* outside a stable at the Convent in *Korakies*. 2 ♀ *sergenti* were caught at other times in this area. The village of *Kalyves*, south of Suda Bay, was negative.

The village of *Orthuni*, about 18 kilometers southwest of Canea, had been sprayed in previous years but the 1948 treatment had been delayed on account of bandits. On 22 September just before the village was sprayed, houses were negative, with the usual history of sandfly abundance before the first DDT treatment. A number of enormous hollow olive trees within 100 meters of houses harbored anophelines but no sandflies. A week after the houses were treated the same trees no longer harbored mosquitoes.

On a visit to certain oriental sore centers in the District of Rethimnon (see below) 15-18 October, Mr. Balodimos searched for sandflies both by day and in the evening. No sandflies were found in houses. An evening search for an hour after dark in the narrow streets of Archondiki was likewise negative. Of the seven villages visited, all had been sprayed in 1946, 1947 and 1948 except Maroulas, sprayed only in 1946. In the latter there were complaints that bed-bugs and a few sandflies had made their reappearance, but no sandflies could be found on searching several houses and a stable. A suburb of Iraklion, Poros, had been sprayed in 1946 but not since. One sandfly (damaged in mounting) was caught in a house, while an early evening search was negative. A number of large

caves had been dug as airraid shelters and had been occupied by squatters ever since the war. The caves had never been sprayed but no sandflies were found.

While in eastern Crete, Professor Hadjinicolaou visited a village which had never been sprayed, *Adiskari*. Sandflies were moderately abundant along with normal household pests. A sample caught 6 October in one or two houses consisted of 8 ♀ *papatasi*, 1 ♂ 1 ♀ *sergenti*, 1 ♂ *chinensis*.

Phlebotomus in the District of Canea and also in other parts of Crete is clearly at a very reduced level of abundance compared with that prevailing in 1934 and 1935. The many solidly consistent reports of local residents leave no doubt that a sharp decrease occurred in 1946, and indeed began with the first actual spraying of the premises of the individuals concerned. This applies to all villages we visited and in part to the city of Canea. Even where the individuals' houses were not treated, a decline was reported as having occurred about that time and was associated by the people with the DDT which was being applied, usually somewhere in their neighborhood. The reports of the people must be considered as relating chiefly to *papatasi*, since this species was the dominant one.

Unlike Attica where we had several unsprayed areas for comparison, in Crete we were hampered by the lack of any available area, rural or urban, which had never had at least some DDT at one time or another. The closest approximation to the latter was a small group of houses in Agios Ioannis, where daytime searches consistently yielded a small number of sandflies. While these houses had never been treated, they lay between and within 100 meters of two groups of houses which had had residual DDT in 1946. The presence of a few or no sandflies during 1948 in places where no DDT had been applied since 1946 or 1947 was a phenomenon we encountered several times, as in Agios Ioannis, the town of Paleochora, and the villages of Orthuni, Agios Marina and Maroulas. Without attempting to draw any conclusion on this point, there may be mentioned the long-term effect observed in Peru (3) where, however, outdoor resting and breeding places were sprayed as well as the houses.

In the villages sprayed for three consecutive seasons sandflies in all cases were extremely scarce. The complete absence of sandflies in daytime searches was to be expected on the basis of all previous work. Those evening observations which we were able to make also indicated the scarcity of sandflies out-of-doors within the treated area. The evening searches in villages are admittedly too few in number to clinch this point, particularly in view of the importance of *major* as the supposed vector of kala azar and the fact that this species rests in houses far less than *papatasi*. The true abundance of *major* has been revealed only when night observations have been included (10), if indeed we are entitled to speak of the true abundance of this species as ever having been measured. The process of catching sandflies "on the move" out-of-doors is hardly to be compared with finding a species in its favorite resting place, such as *papatasi* in houses. Whereas 80-120 *major* represent the highest catches in an evening's work (10), an equal number of *papatasi* can often be caught in a single room. However, in Agios Ioannis the evening observations amply showed that *major* had suffered along with other species.

The effect of the aerospray of Canea in late November 1947 is problematical. Sandflies would have been scarce at that time in any case. The vast majority of the overwintering larvae would already have been in their breeding places and thus presumably beyond the reach of any such treatment.

LEISHMANIASIS

Kala azar. There has been a striking decrease in kala azar in Canea since 1934 and 1935. The one clear feature of the situation is that DDT had nothing to do with bringing about this decline. Dr. Papantonakis and other medical men attributed it to a decrease in dogs, and indeed there is good correlation of the two phenomena. The destruction of stray dogs on account of rabies was begun in 1933 with the result that the investigators of 1934 and 1935 had to deal almost exclusively with house dogs (in 1934 (10), 9 out of 50 infected with kala azar; 1935 (11), 42 positive out of 643). In 1938 Dr. Papantonakis, stimulated by the investigations of 1934 and 1935, undertook the examination of all dogs in Canea and vicinity by means of the formol-gel test (23). 229 dogs out of 1115 were found positive and were destroyed. We understand that the total of dogs examined in the entire campaign, which was continued after the published report, was about 3000. During the occupation, 1941-1945, the shortage of food reduced the city dogs nearly to the vanishing point, with a few "working" dogs surviving in rural areas. Dr. Papantonakis kindly made available to us his records since 1939, which he believes include practically all the cases of kala azar in the whole district (Nomos) of Canea (the western quarter of Crete), since the drugs for treatment were available chiefly through Government sources. For the ten years 1939 to 1948 inclusive the respective totals of kala azar cases per year were 10, 10, 13, 9, 10, 3, 2, 5, 4, 6. In our survey we learned of no case not on his list. The situation in Agios Ioannis (Figs. 4, 5) is perhaps the most striking of all. This small section of Canea, comprising about twenty city blocks, used to furnish most (about 35 cases annually) of the kala azar of the whole district. Only two cases are known from Agios Ioannis in the five years 1944 to 1948.

Since the war the dogs have increased and are apparently approaching normal, but no surveys have been made either as to their numbers or infection rate. As has been shown, *Phlebotomus* is rather scarce at present, the decrease dating from the introduction of DDT in 1946. The role which the sandfly reduction may have played in keeping kala azar at the reduced level which it reached before and during the war is difficult to estimate in view of the gaps in our knowledge of possible reservoirs other than dogs or man, and the absence of any information about local, long-term cycles of kala azar incidence.

The Ministry of Public Health furnished us a summary of the cases of kala azar reported from 49 districts or health centers, covering most of Greece for the years 1939 to 1947 inclusive, but at the same time pointed out that the reporting of kala azar is neither complete nor reliable. For what they may be worth, we cite the respective totals for these nine years: 294, 241, 137, 126, 93, 112, 159, 145, 121. The slight decline which these figures indicate for 1947, which would be the first year in which any effect of DDT could be expected, is obviously not

significant. The variations from year to year in the figures from the individual reporting centers are rather great. If it is assumed that the errors in this compilation would tend to be of the same order of magnitude for any given district, one gains the impression that the epidemiology of kala azar in Greece may resemble that of North China (24) where individual villages may have a number of cases over several years and then have none at all for some time. Adler and associates (10) cite as one of the main characteristics of Mediterranean kala azar its stability, with no annual fluctuation in the number of cases. While this may be true for well studied areas such as Sicily, Malta and North Africa, it has not been demonstrated for Greece. In the case of Canea, we are not convinced that the increase of diagnosed cases from about eight cases per year in 1923-1930 (10, 22) to 40 or 50 annually in the next few years was entirely due to the establishment of the Hygiene Center with facilities for diagnosis. In any case, the only available long-term data for Greece in which we have confidence are those for Canea gathered since 1931.

Oriental sore. We know of no exact data on the incidence of oriental sore in Greece, although several intense foci of the disease have long been known, notably those of Crete and Lakonia, in the southern part of the Peloponnesus (25). The common name for the disease in Crete, "Khaniotico," is derived from the name of the city of Canea (Khania) reflecting its local abundance. Most of the cases, however, have been concentrated in the old Turkish quarter, Splanzia (Fig. 3), although scattered cases have also occurred in other parts of the city and in nearby villages. Dr. Papantonakis (22) reported that between 1932 and 1934, 614 cases were treated at the Hygiene Center, but that this represented only a portion of the total since some were privately treated and many did not seek medical aid at all. Malamos (25) mentioned the occasionally epidemic nature of oriental sore in both Crete and Lakonia. Something approaching an epidemic occurred in Crete about 1938, according to information from both Dr. Papantonakis and Government medical officers in Rethimnon and Iraklion. It was apparently most marked in and near the two latter cities where the incidence, in contrast to Canea, is normally low. In Rethimnon we were informed that there had been a "great deal" of oriental sore (one estimate ran as high as 4000 active cases) about that time, but that since about 1941 there had been very little. The village of Archondiki was named as the only present center of the disease in that district. The District Medical Officer of Iraklion estimated that about 1938-1939 there had been a thousand active cases in the city but that the current level would be in the neighborhood of thirty. At the height of this "epidemic" a treatment campaign was initiated and pressed with considerable vigor in the western half of Crete, which was followed by a decline of the disease particularly in the Districts of Rethimnon and Iraklion. We know of no associated sandfly studies.

Splanzia was heavily bombed during the war. Many houses are vacant and there has been apparently considerable reshuffling of the reduced number of residents. However, among the people seen on the streets, the scars of oriental sore are relatively much more numerous in Splanzia than elsewhere in the city.

This was particularly easy to demonstrate among the children who inevitably gather about foreigners engaged in a strange activity, and who would represent a more strictly local sample. Over half of such casual bystanders showed scars. Active lesions were scarce, particularly those which had developed in the last two years.

The records of the Dermatological Section of the Hygiene Center had been destroyed during the war and only those since April 1946 were available. The physician in charge estimated that he had been treating about 1200 cases per year prior to 1946, a number four or five times as great as those presenting themselves for treatment in 1932-1934 (22). His estimate was supported by the first few months for which he had records. The period 27 April to 31 July 1946 gave a monthly average of 120 cases, while the last five months of that year averaged 35. The cases dropped from 114 in June, 75 in July, to 41 in August, a figure never reached again. The monthly average was 26.5 in 1947, and 17 in the first eight months of 1948. In general the records did not distinguish between new and old cases, but in a few brief periods when this distinction was made, about two-thirds were new cases. It was the opinion of this physician and, indeed, of nearly all others we encountered in Crete, that oriental sore had declined sharply in the last two years. They attributed it to the introduction of DDT.

There are other examples of sharply restricted areas of intense oriental sore infection besides the classical one of Splanzia. There are certain "oriental sore villages." One, *Daratzo* (Fig. 8), lies about eight kilometers southwest of Canea, and is the only such village we learned of near Canea. Well over half the children show the scars. In other villages the disease is rather uncommon. From the frequency of the reports we received, two villages near Rethimnon have become notorious for their oriental sore,—*Archondiki*, about 20 kilometers west of Rethimnon, and *Margarites*, 25 kilometers east of that city. This region was visited in mid-October by Mr. Balodimos. *Archondiki*, an old Turkish village on top of a long narrow ridge, with a population of about 500, had had many cases about 1938-1939, with estimates of 100 active cases at one time. No active cases were known at present, with the exception of one of some ten years' standing. Scars were common among the children. *Kufi*, near *Archondiki*, with a population of 350, had had relatively little oriental sore, and it was said that there had been none before twenty years ago. Of 23 pupils in a school, only four had scars. One active case had developed in the past year. In *Megali Episkopi*, a village of 1200, about 15 kilometers west of Rethimnon, the doctor stated that there had been much oriental sore but that he had seen none in the last two years. School was not in session, but two teachers estimated the proportion of scars at thirty per cent, with no new cases among the pupils. In *Maroulas*, east of Rethimnon, population 450, scars were common, with no recent cases discovered. In one school in *Perama*, a large village 20 kilometers east of Rethimnon, 11 of 47 pupils had scars, with accounts of many cases ten years previously. *Margarites*, with a population of 1200, like *Archondiki*, is an old Turkish town situated on a long ridge. In two school rooms, 24 out of 35 and 45 out of 50 had scars. There were five active cases, one or two having developed within a year. The decrease in active cases was attributed both to the mass treatment of 1938-1939 and to DDT.

In *Orthes*, a village of about 300, near Margarites, four out of 15 pupils showed scars. One active case had developed during the summer. The village doctor cited the decrease of new cases at the time of the mass treatment and had also noted a further decrease after DDT.

In seeking some correlation of intense oriental sore infection with the type of village and its topography, it may be noted that most of the above were old Turkish villages, located on a ridge or hillside, with stone houses larger and with more massive walls than those of recent construction, crowded along narrow streets. Of the villages near Canea, Daratzo (Fig. 8) is also of this type. It is an old Turkish village located on a low rocky ridge, in contrast with nearby villages (Figs. 2, 7) with little or no oriental sore which, whether of Turkish construction or not, are on more nearly level ground or are composed of houses scattered about among olive groves and cultivated fields. Much of Splanzia itself is on a rocky eminence overlooking the harbor.

Previous investigators have pointed out the contrast between Splanzia with its crowded masonry structures (Fig. 3) and few gardens, and the foci of kala azar in the newer parts of the city. The latter are composed of small houses, practically all with gardens (Figs. 4, 5). There is no obvious contrast, however, between the kala azar centers and adjoining non-kala azar sections. In the past few years the kala azar cases have been so few and so scattered both in the city and in villages, that it is impossible to speak of any present focus. Adler *et al.* (10) found good correlation between *P. major* and kala azar, *P. sergenti* and oriental sore, in the foci which they studied. There still remain to be determined, however, other factors underlying the differences between centers which are endemic for one or the other disease and those which are not, notably the types of available breeding places which favor certain species over others, and the possible reservoirs other than dogs or man.

ITALY

A brief visit was made to Italy and Sardinia 14–30 August 1948.

Abruzzi. Dr. A. Corradetti, of the Istituto Superiore di Sanità, Rome, has in progress a large-scale experimental project for the control of oriental sore in the Province of Teramo in the Abruzzi. His survey in 1948 (26) showed that of over 28,000 persons nearly 3 per cent had active cases, with about 21 per cent showing the scars. In an area of about 200 square kilometers between the Tordino and Vomano Rivers and extending about 20 kilometers inland from the sea, practically every building has been sprayed inside, walls and ceilings, with 2.0 grams of DDT per square meter. The experimental area is surrounded on three sides by territory equally heavily infected. Spraying was begun 6 June 1948 and was about completed the middle of August. It was recognized that a pre-season application would have been desirable but causes beyond Dr. Corradetti's control had prevented it. At the expenditure of no little effort, Dr. Corradetti made arrangements for a visit to the region and was fortunately able to act as guide himself.⁷ The trip was most ably organized and in the four days 16–19 August

⁷ Our warmest thanks are due not only to Dr. Corradetti but also to Dr. Amalfitano, Medico Provinciale of Teramo, and to a number of other members of the Government and

we were able to see the work in progress and make sandfly observations both by day and by night in treated and untreated areas. Our notes and identifications were placed at Dr. Corradetti's disposition and are being included in his report, a typescript copy of which he has kindly forwarded. With his permission certain observations are repeated here.

The dominant sandfly of the endemic area in the Abruzzi has been known for some time to be *P. perfiliewi* (27). This species has been generally supposed to be the local vector of oriental sore (28).

Untreated area: South of the Vomano River daytime searches in several isolated farmhouses showed sandflies to be abundant in both houses and stables, but particularly in stables. Identified samples of house catches: 1 ♀ *perfiliewi*, 3 ♀ *papatasi*, 1 ♀ *pernicius*; stable catches: 2 ♂ 14 ♀ *perfiliewi*, 1 ♂ 4 ♀ *pernicius*; two caves in a gravel pit: 9 ♂ 7 ♀ *perfiliewi*, 1 ♂ *pernicius*.

The city of Ortona is apparently south of the endemic zone. Sandflies were not abundant. 5 ♀ *papatasi* were caught in one house. A sheep stable south of the city was negative.

Evening searches were made at two farmhouses on the road from Roseto to Atri, and inland several kilometers. At one, while still light at 8:30, a number were caught in the stable (3 ♂ 30 ♀ *perfiliewi*) while a few were seen in the house (2 ♀ *perfiliewi*). Most of the females had fed, probably the previous night. Between 9:30 and 10:00 it was windy, but in the lee of the stable sandflies were numerous and biting in the open (caught: 11 ♂ 17 unfed ♀ *perfiliewi*). Outside the second house near the village of Casole d'Atri, about 9:00 o'clock, when it was beginning to get dark, there suddenly appeared a considerable flight of sandflies, alighting on clothing and also biting freely. It was possible to take them three or four at a time about as fast as the suction collecting apparatus could be operated. In a few minutes 3 ♂ 23 unfed ♀ *perfiliewi* were caught.

Sprayed area: At the hotel in the town of Roseto where we spent three nights, no sandflies could be found inside or outside at night in the courtyard where there were pigs. Sandflies used to be abundant in this hotel. Various sprayed houses in towns and isolated farmhouses were negative, except for one sandfly seen in an unsprayed house in the midst of an otherwise thoroughly treated town, where they had been numerous before spraying. In one farmhouse just before the spray crew began the treatment, 3 ♂ 7 ♀ *papatasi* were caught.

At the village of Castellalto on the evening of 18 August we witnessed an extraordinary demonstration of residual DDT "in action." This village had been sprayed 6 June. From 7:15 to 8:20, while it was still light, no sandflies were found out-of-doors either in the open or in weep-holes of retaining walls. At 8:20, as we paused in a narrow cobbled street, great numbers of sandflies settled on our party of six or eight, comparable in intensity to the flight of the previous evening in Casole d'Atri. A number were caught in a few minutes (14 unfed ♀ *perfiliewi*). This was near a house where we were guests of honor at dinner. At 8:30 just before being seated at the table, a sandfly was noted floating in a water

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pitcher, the first of what soon became a shower of *Phlebotomus* on the table. The sandflies were in evident distress. They were greatly agitated and fell frequently on alighting,—the typical reaction of sandflies after a few minutes' contact with DDT, observed experimentally (1). Those that fell on bare arms or hands sometimes succeeded in biting. Others made frantic but vain attempts, running about and lowering the proboscis repeatedly to the skin or even sleeve. About 9:00 a tour of the large room showed a number of sandflies on the floor and a few on the walls. About 9:45 it was noted that on the high ceiling above a cluster of bright lights suspended over the table, there were a great many sandflies, all in a state of agitation. It was estimated that 400–500 could be seen at one time in the circle of light. By about 10:30 there was only an occasional sandfly on the ceiling or on the table, with stragglers up to 11:30. A total of 102 sandflies (the females mostly unfed) were collected from the table, the assembled company picking them up with toothpicks dipped in wine and passing them to us. An identified sample consisted of 6 ♂ 20 ♀ *perfiliewi*. The total number of sandflies which entered the room must have been well over a thousand.

Our host informed us that similar showers of sandflies had been observed on the table for a number of days after the spraying, but that there had been none in the ten days before our visit. Before the spraying the annoyance from sandfly bites while at table had been extreme. Villagers told us that they were now bitten out-of-doors but that the sandflies "didn't come into the houses any more."

Dr. Corradetti visited Castellalto again 1 September. At the same time and place in the street where we had encountered the outdoor attack, his party finally saw one sandfly after a wait of five or six minutes. In the dining room not a single sandfly was seen in 45 minutes. Our host informed him that there had been a few sandflies for about three days after our 18 August visit and none since. A possible explanation of the phenomenon we observed is that we just happened to encounter a flight representing a generation emerging from eggs laid before the spraying 6 June, 73 days previously. On the evening of 2 September, Dr. Corradetti visited the same place in the untreated area near Casole d'Atri where we had found the heavy flight. Sandflies were even more numerous and his party, after a few minutes of collecting, found it necessary to abandon the place on account of the number of bites they were suffering.

In our experience we have never encountered any early evening flights comparable in intensity to those of *perfiliewi* in the Abruzzi, with man being bitten freely in the open. This point is of immediate importance since it means that many are bitten at an hour before anyone has retired behind the barrier of residual DDT, bednets or other methods of protection. The net result would be the maximum exposure to the sandflies of the region. By the same token, the degree to which house spraying alone can eventually reduce the entire sandfly population within such an area becomes a matter of prime practical importance. Dr. Corradetti's results both in terms of sandfly and disease incidence will be awaited with much interest.

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Pontine Marshes. Professor Missiroli informed us that in the Pontine Marshes sandflies had been very numerous before the DDT-malaria campaign begun in

1944-1945, and that they had been the cause of more popular complaint than the mosquitoes. They have ceased to be a problem.

An early evening search, 29 August 1948, in and around one group of farm buildings near the Lago di Fondi, east of Terracina, was negative.

Sardinia, 23-27 August 1948. Through the kindness of Dr. John A. Logan, Director, and Dr. T. G. H. Aitken, Entomologist, of the ERLAAS anopheline eradication project, it was possible to visit a number of areas in the southern part of the Island. Beginning about the middle of November, every man-made structure in Sardinia had been sprayed during the winter of 1947-1948. While it had been impossible for the ERLAAS staff to pay much direct attention to *Phlebotomus*, it had been a matter of general experience that sandfly annoyance, formerly very great in many places, had ceased.

Dr. Aitken's staff had made house catches of sandflies in several villages north of Cagliari between 22 August and 12 September 1947, principally in Monastir. Four collections in this village totaled 27 ♂ *papatasi* and 55 ♀ of which about half were examined and found to be all *papatasi*.

We made daytime searches in the villages of Muravero and San Vito, about 50 kilometers northeast of Cagliari, in Quarto, just east of the city, and in certain large occupied caves in the northern part of the city. No sandflies were found and most of the residents reported that sandflies had been abundant in former years but that there had been none in 1948. Monastir (sprayed 15 December 1947) was visited one evening about an hour after dark. Three or four courtyards with stables were negative.

Searches were made along the road in the hilly and sparsely inhabited region about 25 kilometers northeast of Cagliari. Culverts (all sprayed) were negative. Rocky crevices and recesses (unsprayed) within half a kilometer from the main road, were negative. These places were of a type which in Peru would probably contain *Phlebotomus*. No caves were found in the region. The lack of sandflies on these uninhabited hillsides is naturally not to be associated with any control measures. Along the road to Sanluri, running through a well cultivated region, recesses in exposed rocky surfaces were searched without success, with the exception of one small cave at the north edge of the village of Villagreca, 30 kilometers north of Cagliari. In this unsprayed cave, 60 meters from the nearest sprayed house, on two different days prolonged search netted a total of ten sandflies: 5 ♂ 2 ♀ *perniciosus*, 1 ♂ *perfiliewi* (the first record, we believe, from Sardinia), and 1 ♂ 1 ♀ *minuta* group. These were the only live sandflies we saw in Sardinia.

These limited observations combined with the reports of the people indicated that sandflies were still at an extremely low level eight or nine months after the winter application of residual DDT.

SUMMARY AND CONCLUSIONS

1. An evaluation in terms of *Phlebotomus* control has been made of the extensive DDT-malaria campaign which has been in progress in Greece since 1946. Observations were made in Attica and Crete throughout the sandfly season of 1948.

2. In unsprayed places the sandfly population was normal in abundance and distribution of species when compared with the reports of previous investigators. Nearly 3000 sandflies were caught and identified.

3. Sprayed buildings were uniformly negative for *Phlebotomus* of any species. Night observations out-of-doors showed sandflies to be at a very low level within sprayed areas. The testimony of the people was solidly to the effect that sandflies had been annoyingly abundant but ceased to bother them indoors after the first treatment, and that there has been little or no annoyance outdoors at night.

4. The reduced sandfly abundance in the cities of Athens and Canea, Crete, which have not been sprayed *in toto*, may be due in part to the peripheral effect of the many treated buildings scattered through the urban areas, and in part to the use of household sprays—factors which have not been measured.

5. A modification of the oiled paper trap is described.

6. The present status of leishmaniasis in Canea is compared with that of previous years.

a. A very marked decline of kala azar had occurred prior to the use of DDT, and was associated with the destruction of infected dogs and the general reduction of the dog population. With the return of dogs to normal, it is difficult to assess the effect of the present low sandfly level because of lack of information about factors such as other reservoirs and normal kala azar cycles.

b. A sharp drop in oriental sore with the development of relatively few cases in the last two years, coincided with the introduction of DDT in 1946.

c. Definite conclusions on leishmaniasis control must await long-term observations in endemic foci.

7. A recently initiated project for the control of oriental sore in the Abruzzi, Italy, was visited. Observations on the dominant sandfly and supposed vector, *P. perfliewi*, were made in treated and untreated areas. There was witnessed the actual destruction of great numbers of sandflies on encountering residual DDT, resulting apparently in the progressive reduction of the local sandfly population.

8. In Sardinia, where every man-made structure has been treated with DDT, houses were negative eight or nine months later, and sandfly incidence was at an extremely low level.

9. On the basis of the present and previously published work, the following general conclusions seem warranted:

a. Treatment of interiors with residual DDT gives immediate and virtually complete protection from sandflies indoors.

b. House spraying alone, in compact communities, with an annual, preferably pre-season, treatment, eventually reduces the *Phlebotomus* population within the sprayed areas to near the vanishing point.

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PLATES 1-4

PLATE I

FIG. 1. VILLAGE OF MELISSIA, NEAR ATHENS

Small houses widely separated in the midst of vineyards and cultivated fields.

FIG. 2. VILLAGE IN CRETE

Houses of massive Turkish construction, crowded together, the surrounding rolling or hilly terrain with olive groves and cultivated fields.

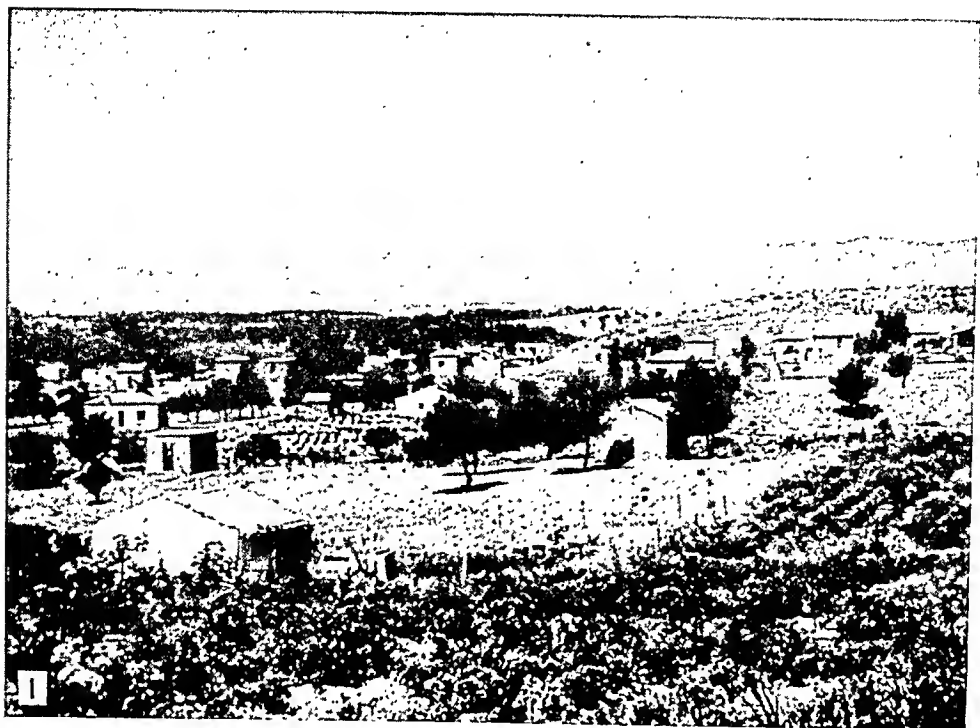


PLATE I

PLATE II

FIG. 3. CANEA, CRETE

Narrow street in Splanzia, the old Turkish quarter; few gardens, little vegetation; an intense focus of oriental sore.

FIG. 4. CANEA

Agios Ioannis, a small section at the edge of the city; practically all houses with gardens; formerly an intense focus of kala azar. Note contrast with Splanzia (Fig. 3).



PLATE II

PLATE III

FIG. 5. GARDEN IN AGIOS IOANNIS

FIG. 6. SANDFLY TRAPS, MADE OF PAPER TACKED TO WOODEN FRAMES AND SMEARED WITH
CASTOR OIL; CAVE NEAR CANEA

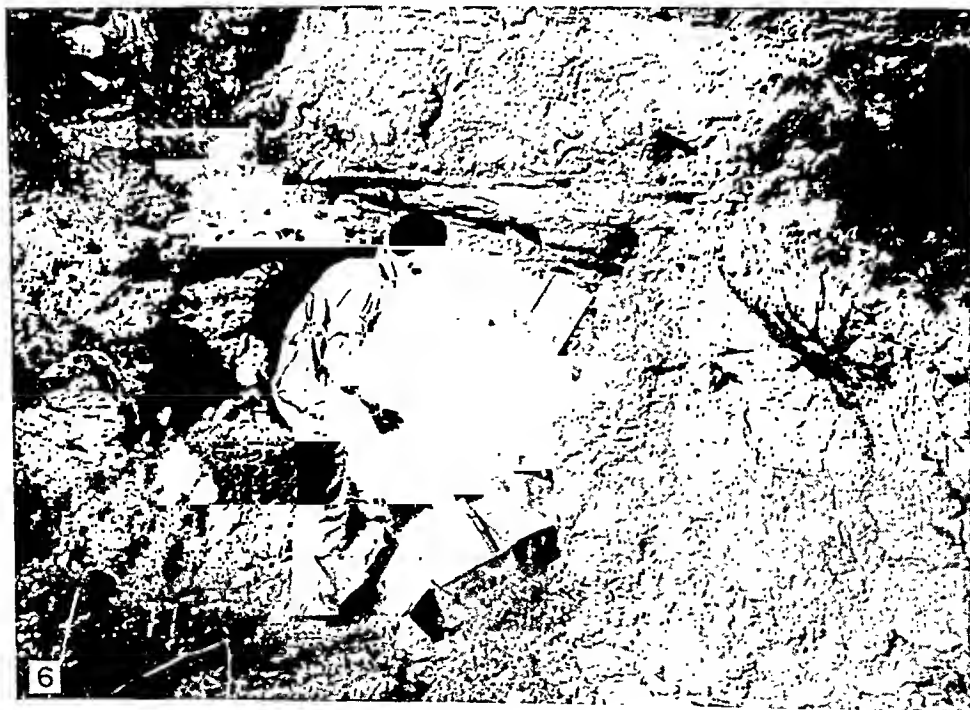


PLATE III

PLATE IV

FIG. 7. VILLAGE OF PARIGORIA, NEAR CANEA, WHERE KALA AZAR HAS
OCCURRED SPORADICALLY

Small groups of houses scattered among vineyards and olive groves.

FIG. 8. VILLAGE OF DARATZO, AN ORIENTAL SORE FOCUS NEAR CANEA.

An old Turkish village on a low rocky ridge; road leading down from side of village to
surrounding olive groves.

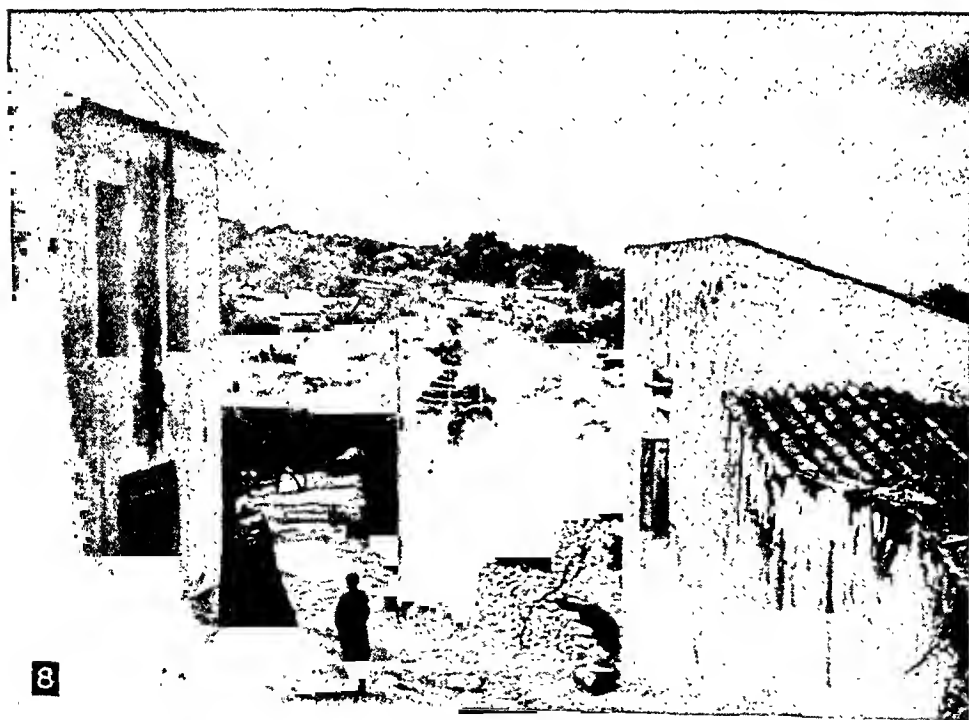


PLATE IV

THE FIRST FIELD TESTS OF RECORDED MOSQUITO SOUNDS USED FOR MOSQUITO DESTRUCTION^{1, 2}

MORTON C. KAHN AND WILLIAM OFFENHAUSER, Jr.³

INTRODUCTION

As a result of many years of close observation of mosquitoes in the field and of mosquitoes maintained in our laboratories, it became evident that there must be some purposeful means of communications among mosquitoes. A single mosquito had often been observed to dart suddenly toward another mosquito over a relatively long distance; this flight activity resulted in copulation. Several physical stimuli such as sight, sound, smell, heat, etc. might individually or collectively cause such action.

In the light of observations and experiments reported some 30 and more years ago, it seemed reasonable to investigate sound as a possibility. The antennae of male mosquitoes is heavily-haired, and it was reasonable for us to suppose that the antennae might act as part of a receptor system for sound transmitted by females. The antennae of female mosquitoes is sparsely-haired; this seemed to suggest that male sounds might be of lesser importance biologically than female sounds.

In a letter to the London Times on Oct. 29, 1901, Sir Hiram S. Maxim (1) told of installing a dynamo at the Grand Union Hotel in Saratoga Springs, N. Y. in 1878, at which time the whine of the brushes on the commutator attracted a very large number of male mosquitoes and very few females. He added in his letter that he had written the observation as a note at the time it occurred and submitted it to a scientific magazine; his note was returned with the editorial comment that it was too stupid to publish.

In about 1900 F. C. Pratt (2) told L. O. Howard that he could distinguish between *Anopheles quadrimaculatus* and *Culex pipiens* by sound alone, and also could distinguish males from females as the pitch of the male sound is higher than the female. In reporting Pratt's observation, Howard mentions that the difference between the note of *Culex* and the note of *Anopheles* becomes at once perceptible by confining the respective insects under gauze in a breeding jar. The note of the *Culex* is higher in pitch, and that of the *Anopheles* is several tones lower and not so clear.

Mr. A. DeP. Weaver (3), an electrical engineer of Jackson, Miss. wrote that while engaged in some experiments in harmonic telegraphy in which a musical note of a certain pitch was produced by electrical means, he was amazed to find that when the note was raised to a certain number of vibrations per second, all mosquitoes, not only in the room where the apparatus was, but also from other

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parts and from outside, would congregate near the apparatus and would be precipitated from the air with astonishing force, striking their bodies against the apparatus. He states that he therefore covered a large surface with sticky fly-paper and after sounding the note for a few seconds captured all the mosquitoes in the vicinity. He then devised an apparatus to electrocute them. An alternating current of high potential was passed and when the note was sounded the insects precipitated themselves against the screen and were immediately electrocuted. Howard commented at the time that "Mr. Weaver, unfortunately, does not state whether males only were captured in this way."

"The Mosquitoes of North and Central America and the West Indies" by Howard, Dyar and Knab (Carnegie Institution of Washington, 1912) contains not only the foregoing but also much other information on the subject of mosquitoes and their sounds. The extracts cited have a direct bearing on the work reported here. In general, it can be said that the early observations, made without the very material aid that modern apparatus can give, were correct and have been confirmed by the authors. The important observations cited above are repeated here because the reference is out of print, and is not generally available.

Howard considered the subject of mosquito sounds to be of such importance as to summarize at some length all the existing literature on the subject. This included information (somewhat inaccurate due to the lack of satisfactory measuring equipment) on the pitch of the notes produced by mosquitoes of different species under different environmental conditions; the data was presented in the form of notes on a musical staff. This ingenious mode of presentation still has some merit.

Mr. J. W. Capstick (4), Fellow of Trinity College, Cambridge made some frequency measurements for Nuttall and Shipley who were interested in the subject. Capstick compared the sound from his subject mosquito with that of tuning forks, a very practical though crude method that still has merit in determining pitch with very simple apparatus. Nuttall and Shipley reported that they were not certain in the case of *Anopheles quadrimaculatus* whether the fundamental tone was 440 cycles per second as the sound had a very strong tone at 880 cps which overshadowed the faint tone of 440 cps.

The shortcomings of the early acoustic work upon mosquito sounds was accurately judged by Howard (5). He states "The exact musical note of the different species seems to have been investigated only in a few cases. Marked differences have been found in the notes of different species. Both sexes emit a sound but there is a marked difference, the note of the males being much higher than that of the female. There is also a slight difference in the note of fed and unfed individuals of the same species." To the authors it seemed reasonable that the advances in recent decades in the electrical recording, reproduction, and analysis of sound should be capable of providing confirmation or denial of these early observations. It also seemed reasonable to think that if the observations proved correct in the main, sound would make possible another useful means of mosquito control and of mosquito classification.

This study deals with a field experiment conducted during the summer of 1948 in Cuba. Recordings were made of single *Anopheles albimanus* female mos-

quitoes; these sounds were reproduced in the swamp from which the female mosquitoes came, through a loudspeaker that was enclosed within an electrically-charged screen. Male *Anopheles albimanus* mosquitoes in quite large numbers were attracted toward the loudspeaker and were electrocuted by the screen. The experiment was performed under controlled conditions using apparatus and methods that can be readily duplicated by skilled personnel. It is believed that this is the first time that a sound-baited trap has been used successfully in an actual swamp (and *not* indoors) for catching mosquitoes.

THE CUBAN EXPERIMENT, 1948

In reviewing the early literature critically, and in checking reports of the effects of sounds upon the behavior of mosquitoes, the impression is gained that technical and non-technical reports alike were characterized by a common failing; the reports are inexact or incomplete. Unfortunately it is practically impossible to attempt to duplicate most experiments as reported. Howard (6) implied that this failing was serious when he wrote: "The exact musical note of the different species of mosquitoes seems to have been investigated only in a few cases."

As the characteristics of the sounds of the chosen mosquitoes (*Anopheles albimanus*) were not precisely known, it was decided that the procedure most likely to succeed in sound trapping mosquitoes was to record the sounds of single female mosquitoes of the desired species found in the Husillo Swamp, and to play back these sounds on a loudspeaker in the same swamp for the purpose of catching the males of the species. An experiment of similar kind conducted by us in the laboratories at Cornell University Medical College with laboratory-raised *Aedes aegypti* had been successful in 1947; it seemed reasonable to think that if the experiment was successful in the laboratory it might be successful in a swamp under natural conditions. Fig. 1 is a photograph of male mosquitoes in a test cage in the laboratory before any sound was emitted by the one-inch loudspeaker shown on the right. Fig. 2 is a photograph taken almost immediately (in a matter of seconds) after the sound from a recording of a single female *Aedes aegypti* mosquito was emitted by the loudspeaker. It will be noted that in Fig. 1 the mosquitoes are flying aimlessly about, while in Fig. 2 they are seen to congregate about the loudspeaker. Tests with the same arrangement in the laboratory had indicated no spontaneous response of a male to an oscillator tone of sine-wave form. When the experiments are conducted during that part of the day when the species is normally very active, the male response to a recorded female call is usually as certain as the sound obtained by pushing a doorbell button.

The recording equipment was set up in the School of Medicine of Havana University. A number of disk records of single female *A. albimanus* mosquitoes were cut. Mosquitoes were provided periodically by the Carlos Finlay Institute from the Husillo Swamp where the Institute has maintained a cattle trap for a number of years. An important reason for selecting this area for a test was that the catches show a consistently high percentage of *Anopheles albimanus*, the species thought responsible for most malaria transmission in Cuba.

The sounds of the female *A. albimanus* are significantly different from all other mosquitoes that we had previously recorded. There is a marked warble of its tone that occurs at a very slow rate (5 or less per second) and the warble amplitude, (as the human ear judges it) seems quite large compared with most other species. It seems similar to the bad "wow" (speed variation) produced by an enlarged or broken center hole of a commercial 78 rpm disk record. The only mosquito showing similar sound characteristics is the *Taeniorhynchus africanus* that we recorded in Nigeria, West Africa in 1947.

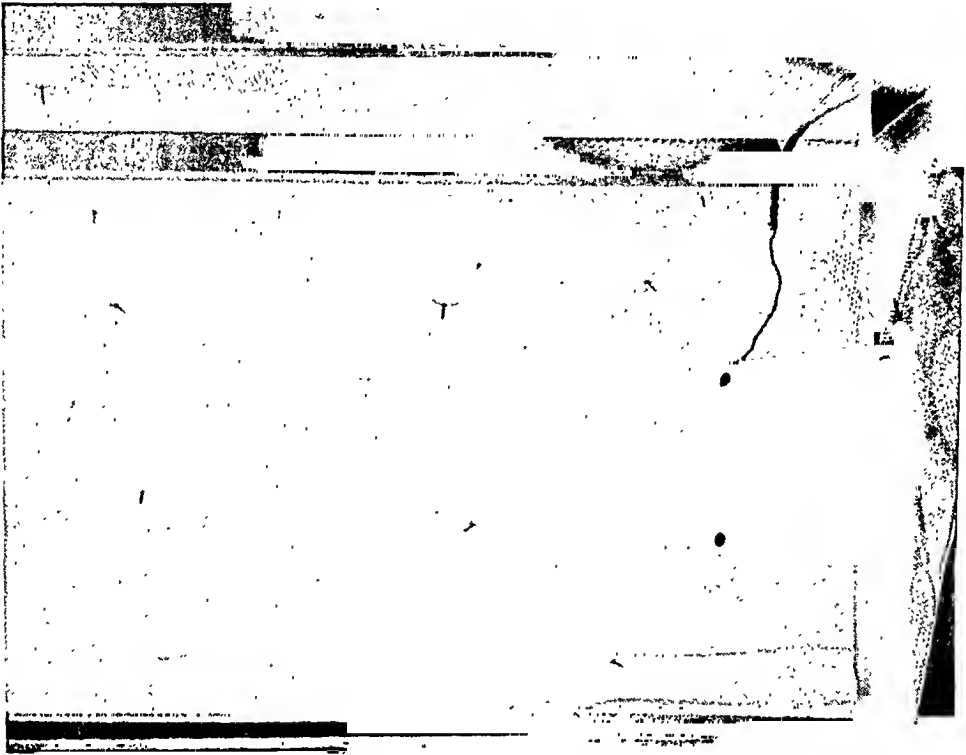


FIG. 1. MALE *Aedes aegypti* MOSQUITOES IN A LABORATORY CAGE BEFORE SOUND WAS
EMITTED BY THE 1-INCH LOUDSPEAKER SHOWN ON THE RIGHT
(Courtesy LIFE Magazine)

As it is unnatural for a mosquito to call constantly for hours at a time, it was necessary to select a suitable section of one of the recordings and re-record (electrically copy) it to make it suitable for use in the swamp test. The selection was made on the empirical basis that the sounds of lowest pitch as judged by the human ear are the most effective as mating calls; this had been found to be true in laboratory work such as the laboratory flight test previously described. The sound selected ran about 7 seconds and a spacing of some 5 seconds or so was allowed before this chosen sequence was repeated. As the playback turntable was a commercial record player (Webster #156 78 rpm automatic changer equipped with a General Electric variable reluctance pickup), a 12-inch Presto acetate disk was recorded in conventional manner with sufficient repetitions to

fill the record completely. This was done in order to make it possible to have the record continuously repeated when using it in the swamp.

Fig. 3 is a sketch of the test layout in the Husillo Swamp. The shack in which the automatic record player and the amplifiers were located is about 68 yards from the Husillo Road, a rough single-width dirt road. A loudspeaker surrounded by its electrically charged screen (Fig. 4) was located about 5 yards behind the shack. The loudspeaker was pointed in the direction of a backwater of the Husillo swamp which was some 60 yards beyond the loudspeaker. About 5 yards to the

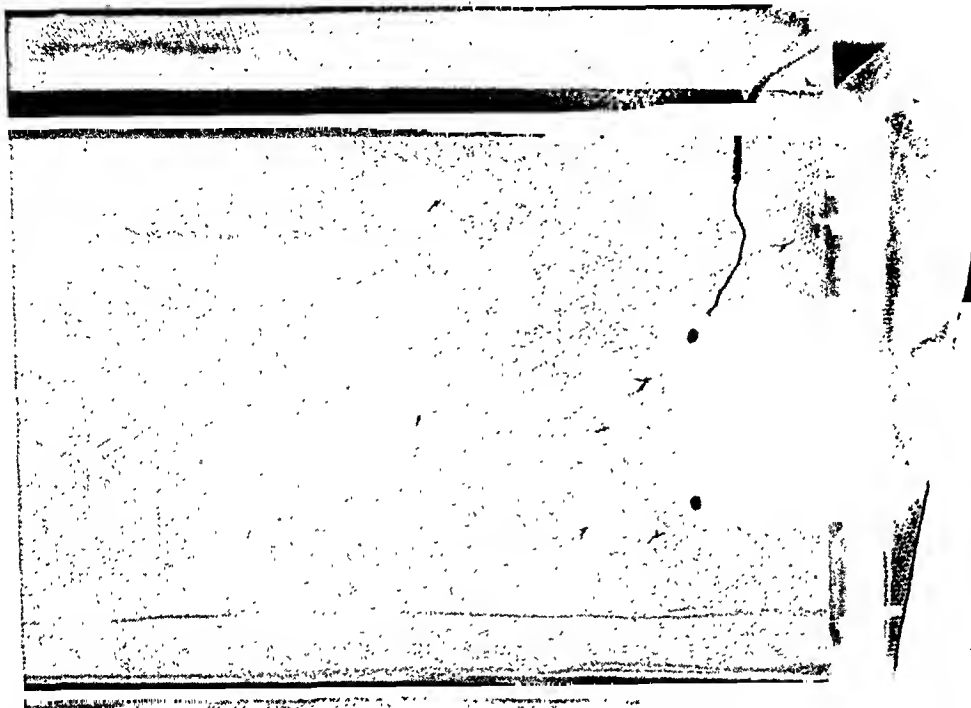


FIG. 2. SAME MOSQUITOES ALMOST IMMEDIATELY AFTER SOUND FROM THE LOUDSPEAKER WAS EMITTED

The sound source was a disk record of a single female of the same species.
(Courtesy LIFE Magazine)

left of the loudspeaker was a banana patch about the same distance to the right was a yucca patch.

The electric screen used to enclose the loudspeaker was 6 ft. high by 3 ft. by 3 ft. It was made especially for us through the Detjen Corporation of New York City. This electric screen is of excellent mechanical and electrical design and seems suited to operation under humid conditions such as those found in the tropics. It has a series of parallel wires with about $\frac{3}{8}$ inch separation between centers; alternate wires are oppositely charged so that substantially the same voltage appears across any adjacent pair of wires. Because of the high moisture condensation occurring in the swamp, it was necessary to elevate the screen frame on clay bricks to reduce electrical leakage to ground.

When the loudspeaker was first turned on and the high voltage simultaneously applied to the screen, it was dusk (near 7 PM local Cuban time) and over 500 electrical discharge flashovers were observed in a 20 minute period. As each electrical discharge should kill at least one mosquito, we expected to find some 500 mosquitoes on the green sheet stretched on the ground underneath the electric screen. When the high voltage was shut off, less than 25 mosquitoes were

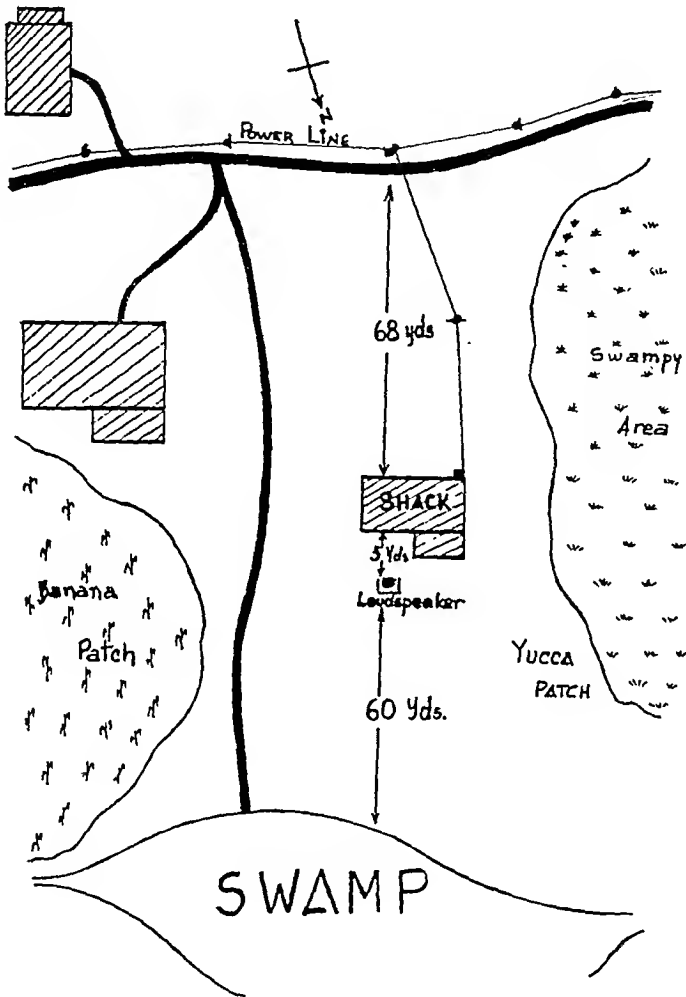


FIG. 3. SKETCH OF THE HUSILLO SWAMP TEST LOCATION
The record player and the amplifiers were located in the shack.

found. Investigation showed that the high voltage transformer supplied (General Electric 53G832) that had been made especially for use with the screen failed to kill mosquitoes. It was necessary to resort to the special arrangement shown in Fig. 5 to make certain that a mosquito coming in contact with the screen would be killed. The arrangement shown has the advantage (essential for research purposes) that it kills mosquitoes without significant mutilation or burning that might interfere with the accurate determination of species and sex. Unfortunately the arrangement is definitely lethal, and is likely to kill a human being



FIG. 4. NAVY BEACHMASTER LOUDSPEAKER USED TO REPRODUCE SOUND IN HUSILLO SWAMP

(Note the electrically-charged enclosure surrounding the loudspeaker)

A. Setting up electric screen and Beachmaster. Green sheet used for actual calling experiments. Dead insects fall into sheet.

B. Apparatus in place for calling experiments. White sheet used to obtain photo near sunset.

The Western Electric X-66468-C2 Beachmaster was provided through the courtesy of the Department of the Navy, U. S. Government. This was manufactured as part of an announcing system for directing the landing of troops during the recent War. This loudspeaker is especially useful as it is very rugged, is resistant to deterioration due to moisture, fungus, etc., and has high conversion efficiency in the frequency range of interest.

or animal that may come in contact with it. In the practical application of mosquito destruction equipment where research is not involved, it should be possible to design a non-lethal killing means that is certain to destroy mosquitoes without harming human beings or farm animals. Fig. 6 is a time exposure photograph of mosquitoes being electrocuted at the screen.

Something should be said at this point about the relation of the directional radiation characteristics of the loudspeaker to the mosquitoes approaching it. The loudspeaker chosen (Fig. 4) has a radiation angle of 50 degrees in either horizontal or vertical direction about the axis of its center horn. For mosquitoes approaching from the front, it is reasonable to suppose that if the sound volume

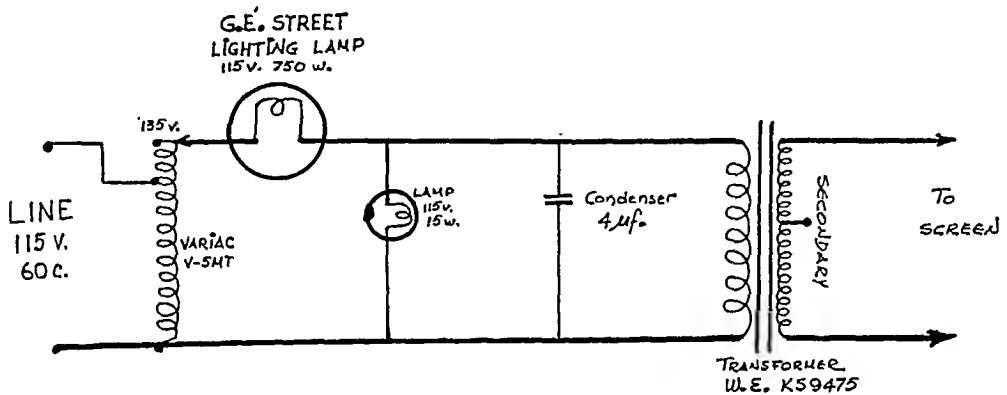


FIG. 5. SCHEMATIC DIAGRAM OF THE CIRCUIT USED FOR MOSQUITO ELECTROCUTION

In actual operation the power line voltage was about 100 v. Connection for the input was made to the terminals for 115 volts; the output was turned up full (nominal 135 volts). The street-lighting lamp operated as a current-limiter, and prevented burnout when a frog etc. might short-circuit the transformer secondary by jumping on the screen. The 15-watt lamp acted as a pilot lamp, and blinked every time the screen was hit by a mosquito or other insect, and dimmed as the street-lighting lamp lit up when the screen was hit by a frog etc. This arrangement permitted a high short-circuit current for a short period of time. The Variac is a standard product of General Radio Co.; the power transformer is made by Western Electric Co. for the U. S. Navy as part of the Beachmaster announcing equipment.

is correct, most mosquitoes will hit the electrically-charged screen in a pattern centered about the axis of the center horn in the manner of a shot scatter-diagram about the bulls-eye of a rifle target. Should the sound volume be increased beyond the optimum, the shots should center about the target rings farther and farther outward from the bulls-eye, the distance outward depending upon how much the sound volume differs from the correct volume. Preliminary rough tests over a volume range from about 15 electrical watts down to 10 milliwatts indicated that bulls-eye centering seemed to occur at plus 10VU (Volume Units) as measured on an American Standard Electrical Volume Indicating Meter (Weston Model 862). This volume level was used throughout the field test in the Husillo Swamp.

Early observations of the first ten days or so indicated that despite the relatively large numbers of mosquitoes caught each night, our sound equipment

would not materially influence the time of flight of *Anopheles albimanus* mosquitoes. To determine their time of flight, we took data on the number of electrical discharges occurring during successive ten-minute intervals. Since a visible and an audible discharge occurs when an insect comes in contact with the electrically-charged screen, a count was made interval by interval. Logs were kept by several observers; one purpose was to check the observations for personal error. At the end of each experimental run, the sheet under the electric screen on which the killed mosquitoes dropped, was carefully folded and examined the next morning not only for the quantity of mosquitoes, but also for the analysis of the catch with regard to species and sex, extraneous insects, their classifica-



FIG. 6. NIGHT PHOTO OF MOSQUITOES BEING ELECTROCUTED WHEN RESPONDING TO SOUNDS FROM THE LOUDSPEAKER

The mosquitoes killed were *Anopheles albimanus* males. The disk record used was a recording of a single female of the same species.

tions, etc. Table 1 is a typical table showing the per cent of the maximum number of discharges occurring during each test time interval and the measured light intensity at the beginning of the time interval.

It is noteworthy that the number-time distribution of the electrical discharges in Table 1 approximates somewhat a normal probability distribution. This general distribution form was characteristic throughout the test; it occurred almost daily in every test run from August 13 to August 31, 1948. Also noteworthy is the tabulation of light level *vs.* the per cent of maximum number of electric screen discharges. This latter suggests a fairly high level of male mosquito activity for this species at light levels as high as 13 candles per sq. ft. This finding would appear to be of interest because of the commonly-accepted idea that *Anopheles*

mosquito activity is a maximum within the rather narrow light level range from 0.1 to 1.0 ft. candle as determined by other methods of observation.

As is indicated in the foregoing table, there was considerable mosquito flight activity (as determined by the number of insects killed in the trap) from the hours of 6:30 PM to about 8:10 PM, with but little flight activity before or after that short period. Since trapping tests were made regularly every day near sundown, the insect catch found on the sheet as a result of each test was held

TABLE 1

Per cent of maximum number of electrical screen discharges resulting from sound-baiting of a trap to catch Anopheles albimanus male mosquitoes

Table 1 shows time vs. light level vs. per cent of maximum number of electric screen discharges on a typical test day.

LOCAL TIME		LIGHT LEVEL	MAX. NO. OF SCREEN DISCHARGES
(PM)			%
	6:30	125	10
	6:40	100	15
	6:50	50	31
Sunset (official)	6:55		
	7:00	13	73
	7:10	1.6	83
	7:20 less than	0.2	100
	7:30	dark	64
	7:40	dark	30
	7:50	dark	21
	8:00	dark	10

Note 1: The time shown is the beginning of the 10 minute interval.

Note 2: Light level is that read on a Weston Master II Exposure meter that was calibrated by Weston. The level was read by pointing the instrument at the clear western sky in such manner as to obtain maximum reading due to sky light.

Note 3: The electric screen discharges are tabulated as percent of maximum number of discharges because the actual number of discharges in the peak interval varied considerably from night to night depending upon environmental conditions. The percent maximum figures, however, repeated night after night, and the figures shown may be considered representative. Screen discharge data is for August 24; light level data for Aug. 27.

over to the next morning for examination. As the catches were quite large often-times running over 1,000 insects and a few times higher it was impracticable to attempt to examine 100 per cent of each catch. A random sample of 100 mosquitoes was examined from each catch and the data shown in Table 2 is based on such sample examination results. Each of the 100 mosquitoes was examined under the microscope for identification as to species (*Anopheles albimanus* or other) and as to sex.

Inspection of the table suggests several conclusions:

1. There is no correlation between the cattle-trap catch and that of the sound-baited electric trap.

TABLE 2
Day to day field data summary

(1) DATE	(2) PEAK DISCH.	(3) CATTLE TRAP CATCH	(4) 6 PM TEMP. °C.	(5) PRECIP. IN.	(6) % <i>M. A. albi-</i> <i>manus</i> TOTAL MOSQ.	(7) % <i>M. A. albi-</i> <i>manus</i> TOTAL <i>A. albimanus</i>	(8) RATIO OF 7 TO 6
19	307	12	27.4	0.01	67	90	1.34
20	100	18	26.7	0.19	72	92	1.29
21	159	23	26.5	4.52		Rainstorm	
22	291	—	28.0	0.98	60	87	1.45
23	276	4	29.0	0	67	92	1.37
24	219	12	28.4	0	64	88	1.37
25	287	13	28.0	0	68	88	1.29
26	110	8	22.5	0.55	62	98	1.58
27	225	14	26.0	0.09	74	92	1.25*
28	133	24	27.7	0.01	74	92	1.25
29	50	—	27.8	0	14	31	2.20 {Control—No Sound
30	145	—	28.1	0	68	93	1.37 {Peak Test
Average.....					68½	91	1.35

* Bottom third of screen disconnected.

Column 1. The date.

- The maximum number of electrical discharges occurring during a 10 minute test interval. (The total number of electrical discharges averaged about 4 to 4½ times the peak number of discharges. As shown in Table 1, the peak occurred quite consistently at 7:15 PM Local Cuban time.
- The female *Anopheles albimanus* catch in the Finlay Institute cattle trap in Husillo Swamp for the previous night.
- The official 6 PM temperature reported by the Cuban Weather Bureau near Morro Castle (National Observatory).
- The total precipitation in inches for the previous 24 hrs.
- The ratio of male *Anopheles albimanus* mosquitoes to all mosquitoes caught.
- The ratio of male *Anopheles albimanus* mosquitoes to total (males plus females).
- The ratio of Column 7 to Column 6.

Explanatory Notes:

1. From the 19th to the 26th inclusive the high voltage was applied to the full 6 ft. height of the screen. From the 27th onward, the lower third of screen was disconnected (to reduce the number of plant lice etc. in the catch).

2. On the 29th a control run was made with the high-voltage on the screen but with no sound issuing from the loudspeaker.

3. On the 30th (during a demonstration for Cuban officials) a peak test was run. The equipment was turned on at 6:55 PM and shut off at 7:30 PM. Electrical discharge counts were made from 7:00 to 7:10 and from 7:10 to 7:20 PM.

2. The numbers of mosquitoes caught in the sound-baited trap are very large compared with the numbers caught in the cattle-trap.

3. The average of $68\frac{1}{2}$ per cent for the percentage of male *Anopheles albimanus* mosquitoes trapped compared with all mosquitoes trapped is high, showed but small variation.

4. The average of 91 per cent for the percentage of male *Anopheles albimanus* mosquitoes trapped compared with all *Anopheles albimanus* mosquitoes trapped is likewise high, and showed but small variation.

5. The control run shows a significantly smaller number of mosquitoes trapped as well as significantly smaller percentages of male *An. albimanus* compared with all mosquitoes, and compared with all *An. albimanus*. (50 compared with over 100, 14 per cent compared with $68\frac{1}{2}$ per cent, and 31 per cent compared with 91 per cent respectively.)

6. The difference between the average percentages and the percentages determined for the peak test of Aug. 30 is small. (Average of 1.37 compared with peak test value of 1.35.)

TABLE 3

The number of hits recorded on different parts of the electric screen during a peak activity period for Anopheles albimanus

TIME	FRONT	TOP	LEFT SIDE	RIGHT SIDE
7:10 PM	89	24	21	48
7:20 PM	94	30	35	43
	183	54	56	91
Grand Total.....				384

7. All figures suggest that the recorded sound calls the desired mosquitoes and has little effect on other mosquitoes.

The literature suggests that the flight habits of some male mosquitoes involve flying at altitudes considerably above 6 ft. To test the effect of the sound-baited trap on such habits, the number of electrical discharges occurring on the top of the electrically-charged screen was tabulated and compared with the number of hits on the front and on the two sides. As will be seen in Fig. 3, a banana grove is on the right side, and a yucca grove on the left. No tabulation was made for the rear of the screen as this panel was not used since it would interfere with convenient access to the loudspeaker located within.

The table suggests that a small yet significant number of mosquitoes dive toward the loudspeaker from altitudes above 6 feet. The number of hits at the front suggests that most mosquitoes fly directly toward the loudspeaker. The comparison of the number of hits on the right side with that on the left suggests that it should be more advantageous to investigate the banana grove more thoroughly as a possible daylight hibernation spot for *albimanus* than the yucca grove. The hits occurring at the top of the screen produced the impression that they were due to small swarms of mosquitoes rather than to the more frequent approach of individual mosquitoes. Although numerous repeat observations

were not made as the information obtained was considered of secondary importance, casual observation at other times confirmed the impression that the data shown in the table is typical.

Some incidental observations appear worthy of mention. On the first night when the loudspeaker was turned on, there was a dramatic procession of tree frogs along a tree frond silhouetted against the western sky; at the end of the leaf they hopped off toward the electric screen where they were shocked. The frogs appear to recognize mosquito sounds for what they are as their jumping toward the screen was fairly consistent when the loudspeaker was operating. Bats and dragon flies also seem to hear the mosquito sounds and recognize them as they hovered about the screen continually; not a single bat or dragon fly was killed or came in contact with the screen. Chameleons were killed frequently in the same manner as the frogs.

The frequent electrical discharges produced a noticeable amount of ozone; its presence did not appear to affect the size of the catch one way or the other. In a catch of electrocuted insects were insects other than mosquitoes. A typical catch included 10 large and small moths, 6 plant lice, 3 beetles, a few large wasps and several small ones. The number of hard-shell beetles that was killed was small compared with the number that frequented the trap. It seems likely that the moisture about the hard shell provides a low-resistance shunt path for the discharge, reducing the likelihood of electrocution.

Quite frequently a small unidentified wasp-like insect with its ovipositor *stinger* was imbedded in the abdomen of a mosquito. These wasps would not ordinarily be seen with the unaided eye, but were clearly evident with a binocular microscope using a 3 \times objective and 15 \times eyepieces. Although this observation may be without significance, it occurred so frequently that it is thought advisable to report its occurrence.

SUMMARY AND CONCLUSIONS

It seems reasonable to draw the following conclusions from the Cuba experiment and its background;

1. Female calls of wild-caught anophelines obtained from a cattle trap such as *A. albimanus* can be recorded, and a re-recorded test record obtained of sufficiently good quality to be effective for sound-baiting a trap to catch males of the same species.

2. The calls which seem of lowest pitch as judged by the human ear are seemingly the most effective for calling the males.

3. The female calls used are not sine-wave in shape but are quite complex, and are not audible ordinarily to the unaided human ear.

4. A test record of female sounds as described in (1) will cause males of the same species to fly in the direction of the loudspeaker. An electric screen (high-voltage-charged) can be placed forward of the loudspeaker for the purpose of killing the mosquitoes that fly toward it. The characteristics of the power supply chosen for the screen can be of such nature as to cause practically no damage or distortion in the killing process that may interfere with the accurate identification of the species and sex of the mosquitoes killed.

5. The mosquitoes so electrocuted are predominantly males (90 per cent) of the species for which the female call is recorded and played.

6. The playing of such a record will also attract frogs, chameleons, bats, dragon flies and other forms of life that prey upon the mosquitoes for food. Certain will be attracted and killed (tree frogs, chameleons); others (bats, dragon flies) will dart around but will not be killed as they do not touch the electric screen.

7. Neither the light flashes produced by the electric discharges, nor the ozone produced seem to have any affect in attracting or repelling the subject mosquitoes.

8. The peak flight time of *Anopheles albimanus* occurred substantially at the same time during each evening of the test (7:15 PM local Cuban time).

9. The numbers of mosquitoes trapped during a peak period of ten minutes exceeded the number of mosquitoes taken from a cattle trap in a week or more.

10. The numbers of mosquitoes trapped each night varied over a fairly wide range from night to night.

11. The playing of such records seemingly will not induce flight activity in male mosquitoes at times when they are not normally active, but will, during periods of normal activity, alter their flight course so that such insects can be killed in a trap.

12. The sound level used in reproducing the records for a sound-baited trap may be quite high compared with the sound level that the mosquito normally utilizes in nature, but it may not be indiscriminately high as there appears to be an upper threshold beyond which a repellant action may take place. Under very quiet natural conditions, the sound level at this upper threshold is such that the sounds can be heard by an unaided human ear some one-fourth of a mile away. It may prove impracticable to use sounds above this threshold for repellant purposes as their unpleasant nature is objectionable.

13. Peak flight time occurred about 20 minutes after official sunset time. At this time the light level measured on a Weston Master II Exposure Meter was about 0.2 candle per sq. ft.

14. Automatic electrical counting of the electric screen discharges is practicable and whenever possible should be used in the collection of counting data in future field experiments. The electrical count should be compared with the count of mosquitoes found on the sheet in the trap.

15. Sound recording techniques may make possible a valuable quantitative sound-activity index for measuring the sound activity of a particular species of mosquito. Such indices may be derived statistically from the curves automatically traced by an instrument such as an Esterline-Angus continuous-writing DC milliammeter energized from a suitable electronic rectifier and filter integrating sound level with time. The electronic rectifier and filter mentioned is energized from the output of the sound recording equipment (capable of recording single insects) that is set to operate continuously over 24 hours or other specified intervals. This method can measure quantitatively regardless of species and sex.

16. Playback of the sound recordings of female mosquitoes to males of the

same species under natural conditions (such as in a swamp) can provide a valuable quantitative activity index based on flight. This flight activity index may be compared with the sound activity index of item 15 for statistical correlation. Playback of the sound recordings of one sex to another may be made in the laboratory to measure the sound response to obtain a sound response index, another potentially useful activity index.

17. Electrical insect-killing methods seem selective, and an arrangement that is effective for one kind of insect, say a fly, may not be satisfactory for another kind of insect, say, a mosquito or a beetle. Important factors are:

- a. Peak voltage
- b. Energy released per discharge
- c. Nature of the current with regard to frequency and waveform
 1. Line frequency alternating current (60 cycles per second or other)
 2. The discharge from a capacitor previously charged to a high voltage (The discharge rate may be quite important).
 3. The discharge of an ultra high frequency generator (functioning at say, 50 megacycles per second).
 4. Other (e.g. a "spike" generator—such as is used in radar).

For mosquitoes where a minimum of distortion is desired due to electrocution, the simplest arrangement using line frequency alternating current is satisfactory although it may be definitely lethal for accidental contact of human beings or farm animals or the like.

18. It is possible that the heavily haired male antennae act as a receptor mechanism for sound emitted by the female.

19. The sounds are substantially species specific, *Aedes* will not respond to *Anopheles* or vice versa.

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THE IDENTIFICATION OF CERTAIN WEST AFRICAN MOSQUITOES BY SOUND¹

MORTON C. KAHN AND WILLIAM OFFENHAUSER, JR.²

INTRODUCTION

In 1947 through the cooperation of Dr. John R. Bugher, Director of the Yellow Fever Research Institute of Yaba, Nigeria, British West Africa, a project was initiated to make recordings of native West African mosquitoes for the purpose of identification of species and sex by sound.

When the project was first discussed, it was recognized that it would be almost impossible to analyze mosquito sounds satisfactorily without first recording them, if the pitfall pointed out so accurately by Howard (1) some 40 years ago was to be avoided. It was necessary, therefore, to make sound recordings with the very best equipment and the very best techniques available. Once permanent records of highest quality were on hand that may be reproduced at will, it then became practicable to utilize whatever methods of sound analysis and portrayal that are deemed best suited.

MATERIALS AND METHODS

Recording Apparatus. Fig. 1 is a block diagram of the recording equipment used. As a practical matter the equipment chosen was to a considerable degree made of non-nutrient (tropic-proof) materials, and was tropicalized so that it could be relied upon to function satisfactorily under conditions of high humidity and frequent condensation. This was very important because the amplification used in recording is so high (approximately 170 decibels).

The microphone chosen was the Western Electric 633A; this instrument has a suitable response range, is quite rugged, and its sensitivity does not change materially even under adverse conditions of use. It is small and convenient to use, and is not affected adversely to an important degree by high humidity and moisture condensation such as we found in Nigeria.

The amplifiers chosen were all Western Electric: one-120C amplifier, one-121A amplifier, and one-124F amplifier were connected in cascade. A "Tee" attenuation pad was connected as a gain control between the output of the 120C and the input of the 121A amplifiers. A 4 decibel fixed loss pad was used between the output of the 121A amplifier and the 600 ohms line-matching input of the 124F amplifier. The output of the 124F amplifier was reconnected for 8 ohms nominal impedance.

The 124F amplifier was used both for recording and for playback. When used for the former, it fed a Presto Model 6N high-fidelity disk recording machine;

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when used for the latter it fed a Western Electric D-173491 loudspeaker. The latter loudspeaker is similar to the commercial Western Electric 728B loudspeaker except that it has a moisture-proof phenolized linen diaphragm in place of the commercial paper diaphragm, and its voice coil has an impedance of 8 ohms rather than the commercial value of 4 ohms.

The rated nominal gain of the amplifier system "wide open" is about 180 decibels. A 6-volt storage battery was used to supply cathode heater current for the 120C and 121A amplifiers; space current for these amplifiers was obtained from a Western Electric 20B voltage-regulated Rectifier. The 124F amplifier was energized completely from the alternating current mains. Western Electric electron tubes were used where applicable because of their marked superiority in performance compared with commercial electron tubes of home radio set or JAN equivalent types.

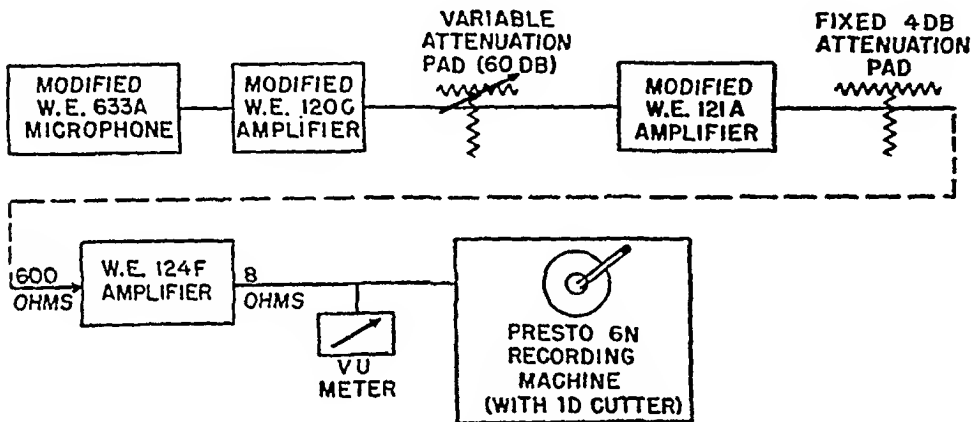


FIG. 1. BLOCK DIAGRAM OF EQUIPMENT USED TO RECORD MOSQUITO SOUNDS

Recording Apparatus Modifications. As the spectral energy distribution of mosquito sounds seems similar in certain respects to that of the human voice, except that high-energy components are located higher in the spectrum and there are more transients, the response-frequency characteristic of the recording amplifying system was arbitrarily "tilted" upward approximately 6 decibels per octave to improve the working signal-to-noise ratio. Characteristic curves and data for the amplifying system and for its component individual amplifiers are given in a paper in press (1949) in the *Journal of the Acoustical Society of America* of the American Institute of Physics by the authors. A description of the modifications found necessary follows:

120C Amplifier. To obtain the maximum grid swing at the grid of the first tube in the amplifier, the input coil of the 120C amplifier was removed and replaced with a Western Electric 285A which latter coil has an impedance ratio of 30:200,000 ohms. To reduce magnetically induced hum to a minimum, a set of three nested permalloy shields were placed around the input transformer. The response-frequency characteristic of the amplifier was "tilted," by modifying the feedback path.

121A Amplifier. The response-frequency characteristic of the 121A amplifier was altered by modifying the feedback path and by reducing the size of the coupling capacitor between the first two stages. No additional magnetic shielding was found necessary for the 618B input transformer.

124F Amplifier. The response-frequency characteristic of the 124F amplifier was not altered as it was also used for playback purposes. The output transformer was reconnected for 8 ohms nominal output impedance; the plate power transformer was reconnected to provide 20 watts rated output that is possible when Western Electric 350B tubes are used in the final amplifier stage instead of commercial 6L6 tubes.

Recording Machine. A Presto 6N Recording Machine was used to cut the records; this was equipped with a Presto 1D high-fidelity cutter (15 ohms rated impedance). A single turn permalloy shield was placed immediately outside the single phase drive motor (approx. $8\frac{1}{4}$ in. diameter) to attenuate the magnetic field radiated by the drive motor.

633A Microphone. As low frequencies are relatively unimportant in mosquito sounds, the low frequency response of the Western Electric 633A microphone was reduced by closing off the air vent from the back of the diaphragm. This is readily accomplished by plugging up the small hole near the terminal connections located underneath the detachable cover.

Overall System. When suitably grounded electrically in a very careful manner, the recording system is stable electrically. As the operating amplification is very high (180 decibels maximum) considerable precautions were taken to insure a minimum of extraneous noise from such sources as microphonic electron tubes and from vibration transmitted to them by the building supports from random sources, and from the vibration of the drive motor used on the Presto recording machine. It was necessary to "float" the whole amplifier assembly on soft cotton batting as the shock-absorbing rubber mountings furnished by Western Electric do not provide sufficient vibration attenuation unaided. It was impossible to use the loudspeaker for monitoring in the same room with the amplifiers due to "feedback howl" that occurred between the loudspeaker and the amplifier tubes; while recording was in progress, head telephones were used for sound monitoring.

Recording Acoustics. As the sounds of mosquitoes are very low in energy level, it was necessary to go to considerable lengths to exclude extraneous sounds from the recording microphone. In nature, the noise generally present under conditions of normal mosquito activity is very high compared with the level of the mosquito sound itself; it is for this reason that the *Aedes*, for example, has been known as the "silent" mosquito. The best microphones and associated electrical equipment presently available to the acoustic art are incapable of discriminating to any important degree against unwanted sounds. The best type of directional microphone, the line microphone, does not hold out much hope as a practical solution as it would require an aiming mechanism comparable to a radar-controlled aiming device to make it useful. Without such an aiming device, its use would be comparable to an attempt to use a rifle to hit a target

with the gun aimed at some unknown aiming point in a different direction. The idea of a radar-controlled tracking device for following mosquito flight is not as far-fetched as it may seem; the recent extension of ultra-high frequency techniques into still higher frequencies now makes radar-tracking of mosquito flight feasible.

The Western Electric 633A microphone chosen for recording is essentially non-directional; it is used within a noise-attenuating enclosure that can be called a "mosquito sound studio". To obtain the desired magnitude of attenuation of noise, it was necessary to attenuate in several stages by "nesting" enclosures within one another. The microphone was used in a small and quiet room; the recording amplifiers and the recording machine and accessories were located outside. To increase the noise attenuation of the room, it was necessary to line its walls with Celotex or similar acoustic wallboard. Within the room was a chest; the microphone fitted to an internal enclosure in which the test mosquitoes were located, was placed inside the chest. When recording, the top of the heavy-walled chest was sealed, and the door to the recording room similarly sealed. Only with all these elaborate acoustic precautions was it possible to obtain a satisfactorily low noise level of airborne noise. The attenuation of the internal chamber and the chest was in the order 60 decibels or more.

Recording Technique. As the sounds to be recorded are produced by mosquitoes without our use of artificial stimuli, a relatively large amount of recording time is required to obtain a sufficient quantity of original recording for each specific test condition. The original records were disks cut at $33\frac{1}{2}$ rpm and at a cutting pitch of a little more than 100 lines per inch; this combination produced the most satisfactory compromise between quality of the record and record cost. Two different types of conventional commercial wire recorders were tried that had been borrowed from the US Navy; these proved grossly inadequate in fidelity and in operational reliability.

To limit the number of costly original acetate disks used, recording is customarily started after the test insect has chirped several times within a few minutes as the sounds are heard in the sound monitoring headphones³. In the *Anopheles*, for example, this occurs fairly consistently near sunset and sunrise; it will occur at other times with other species. Two or three 12-inch disks recorded at the time of maximum natural sonic activity usually provided most of the mosquito sounds thought significant.

The original disks were then edited by playing them back to identify similarities and differences accurately, and to eliminate duplication. *The selected portions* were then re-recorded; since most mosquito sound sequences in which we are interested are of only a few seconds duration, each sequence is repeated several times as a matter of convenience. Should tests of the effectiveness of a particular

³ Sound monitoring during recording is accomplished with head telephones; those used were the Western Electric 509 type. It is ordinarily impracticable to use a loudspeaker for sound monitoring during recording due to the "feedback howl" that occurs between the loudspeaker and the microphone. Although loudspeaker monitoring is possible if the acoustic insulation between the microphone and the loudspeaker is increased considerably, the cost is usually prohibitive.

sound in attracting other mosquitoes be the objective, each sequence is repeated continuously until one side of a disk is filled. When this has been done, it is possible to use such a disk (or a pressing derived from it) on a conventional automatic record changer as a readily-available time-continuous sound source if desired.

Table 1 is a list of the edited records of the African mosquitoes that we recorded. These records are in the form of high quality 78 rpm commercial vinylite disk pressings that were made by processing special masters re-recorded from the original acetate disks that were cut in Nigeria. These disks are different from commercial records in that the playback needle travels from inside to outside, and that each record is not a continuous recording, but is a group of some 7 rings each of which represents a presumably distinctive sound. Considerable effort was necessary to obtain the very high sound quality; the very best in methods and techniques were used not only in the research portion of the process, but also in the commercial manufacture of the finished disks.

When played back, all the sounds recorded proved distinctive; sex and species specific sounds were obtained as judged by the experienced ears of the investigators. As presently available methods of sound analysis diverge over a very wide range of design objectives, it was felt that we should consider seriously Potter's recommendation of the standard of reference; "the human ear—despite the fact that our knowledge of the way it operates is still vague in many respects."⁴

Unfortunately the human ear does not detect sounds as conventional acoustical instruments do, and for the present at least it is necessary to rely upon the observations of investigators experienced in the field of acoustics. Sound analysis by ear and sound analysis by physical instruments lead to anomalous results in the case of mosquito sounds if the nature of the apparent disagreement is not thoroughly understood. The work of Capstick, referred to in the following paper, is a case in point.

Nuttall and Shipley stated that they were not certain in the case of *Anopheles quadrimaculatus* whether the fundamental tone produced was 440 cycles as the sound had a very strong tone at 880 cycles per second which overshadowed the faint tone of 440 cps. The ambiguity is caused by the human ear itself; Capstick⁵ in doing the work requested of him by Nuttall and Shipley, was comparing a mosquito sound that has a very complex relatively non-decaying waveform rich in harmonics, with a tuning fork that has a continuously changing and decaying waveform that is rich in harmonics when first struck, but loses most of its harmonic content in a small fraction of a second. Despite this, Capstick's method of comparing the unknown mosquito sound with a tuning fork, though primitive, is useful provided its limitations are clearly understood. For a modern

⁴ Potter, R. S. "Objectives for Sound Portrayal" Journal Acoustical Society of America, Vol. 21, No. 1, Jan. 1949, pp. 1-5.

⁵ We can surmise that Capstick also had great difficulty in his measurements as he had no assurance that a test mosquito was emitting the same sound at all times when he was attempting confirming observations.

TABLE 1

List of recordings of African mosquitoes (Nigeria, BWA, 1947)

MASTER FACE NO.	NO. OF RINGS	SPECIES	DESCRIPTION
1	7	<i>Aedes aegypti</i>	Female, African
2	2	<i>Aedes aegypti</i>	Comparison-Female African (inner) and Female US
3	3	<i>Aedes aegypti</i>	Comparison-Female African control (inner) Female African test-ex- posed to radioactive phosphorus (2 outer rings)
4	6	<i>Aedes aegypti</i>	Females, African
5	6	<i>Aedes aegypti</i>	Females and males together—Afri- can
6	6	<i>Aedes aegypti</i>	Females and males together—Afri- can
7	6	<i>Aedes aegypti</i>	Males, African
8	7	<i>Anopheles gambiae</i>	Females, African
9	7	<i>Anopheles gambiae</i>	Males—African (6 inner rings) Female and Male together, African (outer)
10	9	1— <i>Anopheles gambiae</i> (inner) 2— <i>Anopheles</i> species (unidenti- fied) 3— <i>Aedes africanus</i> 4— <i>Aedes aegypti</i> 5— <i>Aedes flavicollis</i> 6— <i>Aedes niteocephalus</i> 7— <i>Taeniorhynchus africanus</i> 8— <i>Megarhinus brevipalpis</i> 9—Very small fly-like insects (unidentified)	Comparison, African Females (sin- gle insects)
11	7	<i>Aedes africanus</i>	Female, African
12	6	<i>Aedes flavicollis</i>	Females
13	8	<i>Aedes niteocephalus</i>	Females
14	10	<i>Taeniorhynchus africanus</i>	Females
15	10	<i>Megarhinus brevipalpis</i>	Females

TABLE 2
Analysis of West African mosquito sounds

NO.	SPECIES	DOMINANT FREQUENCY	NOTE AND KEY # ON EQUAL TEMPERED SCALE (A = 440)*	SECONDARY NOTE	NOTES
1	<i>Anopheles gambiae</i>	420	G# - 48	G	Guttural, with pitch constantly jumping back and forth slowly
2	<i>Anopheles</i> sp.	320	E - 44	F	Very piercing, quickly jumping up and remaining there
3	<i>Aedes africanus</i>	530	C - 52	—	Unpleasant with rapid yet small pitch vibrato
4	<i>Aedes aegypti</i>	600	D - 54	—	Unpleasant whine with rapid pitch vibrato slightly greater than #3 and of higher pitch
5	<i>Aedes flavicollis</i>	300	D# - 43	E	Guttural with slight vibrato, with pitch jumping up at end
6	<i>Aedes niteocephalus</i>	500	B - 51	C	Piercing unpleasant whine, with pitch jumping up and down rapidly, tapering off at end
7	<i>Taeniorhynchus africanus</i>	320	E - 44	F	Marked warble sliding between the tones at a rate of about 2 per second
8	<i>Megarhinus brevipalpis</i>	450	A - 49	G#	Marked warble with pitch jumping quickly downward and jumping upward at end of tone beyond the starting pitch

* In the above table, Dominant Frequency is the pitch of the most noticeable tone. The Note and Key Number is taken from Table 3 page 25 of "Proposed American Standard Acoustical Terminology Z24.1 (Feb. 1949)" published by the American Standards Association, Inc.; (4) the note indicated is that nearest the measured frequency. The Secondary Note is the tone that gives the impression of secondary importance. In most cases of pitch change, the magnitude is about one full musical tone; such a condition makes it possible (though somewhat inaccurate) to use the musical staff as a means of notation just as it was used by Howard over 35 years ago.

observer skilled in acoustical observations however, such an arrangement lacks convenience and predictable precision, and where electricity is available, better arrangements are definitely indicated.

The arrangement that we found convenient, simple, and useful includes a pair of head telephones worn by an observer (experienced in acoustical beat-note observation) in which one telephone is energized from a recording of the unknown mosquito sound, and the other telephone energized from a sine-wave audio frequency oscillator, the frequency of which can be conveniently varied for the purpose of matching the pitch of the oscillator to that of the unknown sound. The oscillator used should be reasonably stable in frequency (within 2 per cent), and the harmonic distortion should be less than 2 per cent. Such requirements were met with the Hewlett-Packard 200A Oscillator made by the Hewlett-



FIG. 2. SOUND SPECTROGRAM OF A SINGLE *ANOPHELES QUADRIMACULATUS* FEMALE
(Courtesy Dr. O. E. Buckley and The Bell Telephone Laboratories, Inc.)

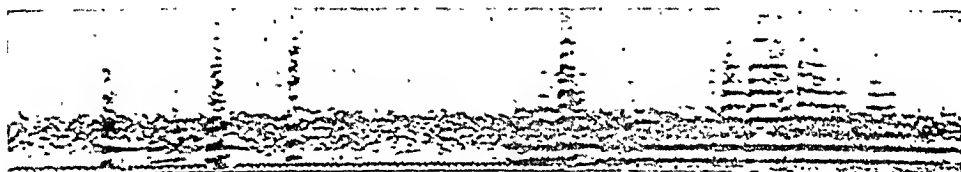


FIG. 3. SOUND SPECTROGRAM OF A SINGLE *Anopheles quadrimaculatus* MALE

This is the sound emitted by the male in response to the female sound shown in Fig. 2.
(Courtesy Dr. O. E. Buckley and The Bell Telephone Laboratories, Inc.)

Further data on sound spectrograms will be found in the Journal of the Acoustical Society of America. "The Sounds of Disease-Carrying Mosquitoes" which appears in the May or June 1949 issue of the same Journal shows the Spectrograms of *Aedes aegypti*.

Packard Company of Palo Alto, California.⁶ The apparent levels were first adjusted to approximately the same values in both head telephones, and the frequency dial of the oscillator turned until a pitch match was obtained; the frequency indicated on the dial was then read. The results of this test are shown in Table 2; it will be noted that there are distinctive classifiable differences among all the mosquitoes shown.

A point worthy of mention in the above table is that the pitch of the *Megar-*

⁶ There are numerous sine-wave audio frequency oscillators on the market that can meet these simple specifications. The authors plan on making similar test observations with other wave forms such as the square wave and the pulsed wave. For the most part, mosquito sounds are pulsed waves similar to radar waves, and there would seem to be no reason to believe that they are not susceptible to similar analysis and mathematical treatment.

hinus brevipalpis, a relatively very large insect, is in the same sonic range as that of the other true mosquitoes which are very much smaller. If the wing size and mass of the mosquito were the only primary factors that determine the pitch of the sound, we should expect the dominant frequency to be less than 100 cycles per second. Although it is possible that the value of 320 cycles per second found by aural matching is in error by one octave due to the peculiarities of the human ear, even that calculated value would be too high compared with the observed value. To resolve such questions with finality requires wave analysis that is best accomplished by electrical means; such analysis avoids the hazard of a detector whose indications may be misleading.

The most suitable analysis equipment is highly specialized, is very costly, and is in very limited supply. This is the sound spectrograph that has been developed in recent years by the Bell Telephone Laboratories Inc. In this graphic apparatus, frequency is portrayed as the ordinate, time as the abscissa, and relative sound intensity by the darkness of the trace. Numerous papers describing the design philosophy of this device and its practical embodiments are to be found in recent issues of the Journal of the Acoustical Society of America of the American Institute of Physics.

Figure 2 is the sound spectrogram of one *Anopheles quadrimaculatus* female. Figure 3 is the sound spectrogram of a male of the same species. Differences are immediately apparent in the traces shown; these differences are also immediately apparent in listening as the pitch of the male seems higher than that of the female. These spectrograms are typical of mosquitoes; analysis of the spectrograms shown and of others indicates the following:

SUMMARY AND CONCLUSIONS

1. All mosquito sounds are composed of a well-defined fundamental in the center of the audio range together with overtones, even in the case of sounds that sound like clicks. In some spectrograms as many as 15 distinctive frequency bands are recognizable.

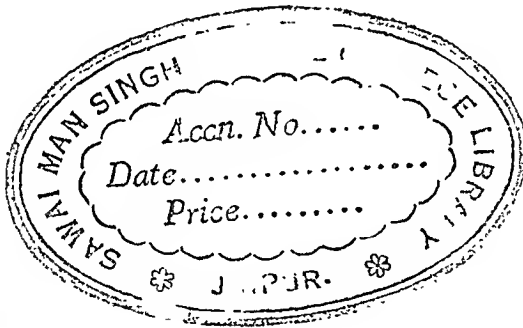
2. All mosquito sounds have vibrato effects. Some species show tones with a single warble rate; others show double warble modulation. A common rate for the low frequency warble is from 2 to 15 cycles per second; the higher rate, when present, is often 5 times the lower. The magnitude of the lower rate may be some 5 to 10 per cent; the higher rate is about one-fifth to one-tenth the lower.

3. Male sounds are usually more broken than the female. In most cases the apparent pitch of the male is higher than the female of the same species despite but a small difference in their fundamentals. Mosquito sounds show a change of pitch with time; some increasing as much as 25 per cent in as little as 0.05 sec.

4. The intensity is modulated in all sounds. In some cases modulation is a complete interruption of an entire band or bands; in other cases, harmonics are varied only, leaving the remainder unaffected.

The spectrograms shown above are presented for the purpose of indicating some of the possible applications of modern electronic methods and apparatus

in the study of the sounds of disease-carrying mosquitoes. They were made available to us through the courtesy of Dr. Oliver E. Buckley, President of the Bell Telephone Laboratories Inc. to whom we owe a debt of thanks not only for providing them, but also for his most welcome encouragement in our application of modern electronic apparatus to our study. It is the authors' considered opinion that the intensive application of such apparatus will make possible the precise, rapid, and simple observation of natural phenomena related to the sounds of disease-carrying mosquitoes and should lead to the more effective control of such mosquitoes and of the diseases that they transmit.



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VIA AIR MAIL

183-193, EUSTON ROAD, LONDON, N.W. 1
31st May, 1949.

Dear Dr. Boyd,

I have just received the *Proceedings of the 4th International Congresses of Tropical Medicine and Malaria*, in which there appears my paper on "The relationship of the Haemoflagellates."

On p. 1113 of this paper 2 lines have been interchanged with the result that this part of the text is incomprehensible.

It is now too late for any alterations but the error could be rectified by drawing attention to it in the *American Journal of Tropical Medicine*.

I should therefore be very grateful if you could see your way to inserting in your journal a note as follows:—

CORRIGENDUM

Proceedings of the 4th International Congresses of Tropical Medicine & Malaria, Washington, 1948.

Paper by C. A. HOARE: "The relationship of the Haemoflagellates" (vol. 2, p. 1110).

Page 1113: line 22 should be transferred to line 39, and *vice versa*.

With kind regards,

Yours sincerely,
DR. C. A. HOARE.

Dr. Mark F. Boyd,
Editor,
American Journal of Tropical Medicine,
615 East Sixth Avenue,
Tallahassee, Florida,
U.S.A.

BOOKS RECEIVED

- PETR WYGODZINSKY. *Elenco Sistemático de los Reduviiformes Americanos*. Spanish-English. Pp. 102, wrap. Monografía No. 1, Instituto de Medicina Regional, Universidad Nacional de Tucuman, Secretaria de Educacion de la Nacion, Tucuman, Republica Argentina, 1947.
- Report by the Dean, London School of Hygiene and Tropical Medicine (University of London) Incorporating the Ross Institute. Report of the Work of the School for the Year 1947-1948. Fifty Years of Tropical Medicine, 1899-1949. pp. 138, illus., wrap. Keppel St., Gower St., London, W. C. 1, England.
- Missão de Combate às Tripanossomíases. Relatório Anual de 1947. Colonia de Moçambique. Imprensa Nacional de Moçambique. Lourenço Marquez. 1948.
- GEORGE R. MOON. *How to Become a Doctor*. Pp. 131, illus. cloth. Philadelphia, The Blakiston Co., 1949. \$2.00.
- N. GOHAR. *Mycoses and Practical Mycology*. With a Foreword by Sir Philip Manson-Bahr. pp. xi plus 234; figs. 134; 4 colored plates. Cloth. Baltimore, Williams and Wilkins Co., 1948. \$6.00.
- BRIAN MAEGRAITH. *Pathological Processes in Malaria and Blackwater Fever*. pp. xi plus 430, colored plate, figs. 22. Springfield, Illinois, C. C. Thomas, 1948. \$10.00.
- ERNEST CARROLL FAUST. *Human Helminthology*. A Manual for Physicians, Sanitarians and Medical Zoologists. 3rd edition, revised. Pp. 744, figs. 313. Philadelphia, Lea & Febiger, 1949. \$10.00.
- MARSTON BATES. *The Natural History of Mosquitoes*. Pp. xv plus 379, 16 plates, figs. 9. New York, The Macmillan Co., 1949, \$5.00.
- Annual Report of the Gorgas Memorial Laboratory, 1948. Letter from the Director*** Transmitting the Twenty-First Annual Report***. 81st Cong., 1st Ses. House Doc. No. 10. Jan. 3, 1949. Washington, D. C., G.P.O.
- EVAN W. THOMAS. *Syphilis: Its Course and Management*. Pp. xix plus 317, figs. 67. New York, The Macmillan Co., 1949. \$5.50.
- JAHAH LAL DAS. *Manual of Hygiene and Public Health with Special Reference to the Tropics*. A Text-book for Medical and Public Health Students. 4th edition, 2 Vol. cloth. pp. x plus 1124, figs. 82. Calcutta, Das Gupta & Co., 1944, 1948. Vol. 1, Rs 7/0 or 8/6; Vol. 2, Rs 8/8 or 13/6.
- HARTWIG, HORMANN. *Serologische Reaktionen und Immunität bei Malaria*. Pp. 107, figs. 42, wrap., Band 5, Schiftenreihe für Seuchenbekämpfung, herausgegeben von Prof. Dr. Med. Robert Kudicke. Stuttgart, Hippokrates-Verlag Marquardt & Cie. 1948. D. M. \$7.50.
- PAUL D. WHITE. *Medical Mission to Greece and Italy*. April 15-June 7, 1948. Pp. 73, illus. Wrap. Boston, Unitarian Service Committee. N. D. Gratis.
- ERWIN KOHN. *Medical Mission to Poland and Finland*. July 1-August 27, 1948. pp. 82, illus. Wrap. Boston, Unitarian Service Committee. N. D. Gratis.

BOOK REVIEWS

MARSTON BATES. *The Natural History of Mosquitoes*. Pp. xv plus 379. Plates 16 and figures 9. Cloth. New York, The Macmillan Company. 1949. \$5.00.

This scholarly and thought provoking review will be welcomed by all those technically concerned with mosquitoes, while others with broader or differing interests may enjoy reading its interesting pages, summarizing what is known of the behaviour of mosquitoes. The natural history of adult mosquitoes is discussed in five chapters, treating of environment, survival and dispersal, sexual behaviour, food habits, terminating with egg development and oviposition. A chapter devoted to eggs is followed by a sequence of four chapters treating of larval environment, physiology and behaviour, biological environment and classification of habitats, which is succeeded by a chapter on pupae. Their relation to other organisms preceeds other chapters presenting their roles as vectors of viruses and plasmodia. The concluding chapters discuss the species problem, classification, distribution, techniques, and the strategy of research. An appendix gives a classified synopsis of mosquito species, as well as a useful bibliography.

Although certain to be widely useful to those concerned with practical problems involving mosquitoes, the work, notwithstanding is primarily directed to students of general biology, in the hope that they will come to appreciate the potentialities of mosquitoes as experimental animals, conveniently available for the study of fundamental biological problems.

MARK F. BOYD

BRIAN MAEGRAITH. *Pathological Processes in Malaria and Blackwater Fever*. With a Chapter on the Parasites of Human Malaria, by Robert H. Black. Pp. xi plus 430. Colored frontispiece and 22 figs. Cloth. Springfield, Illinois. Charles C. Thomas. 1948. \$10.00.

The volume is an attempt to define, from an extensive review of the literature, the basic physiological and pathological processes which determine the reaction of the body to invasion by the malaria parasite and the appearance of blackwater fever, with especial attention to the blood, liver, kidney, brain, spleen, bone marrow, adrenals and heart. The author concludes, from the fragmentary data available, that certain processes, including generalized anoxemia, vascular endothelial damage, together with local and general circulatory changes, the combined effect of which results in the production of tissue anoxia, are common to the development of lesions in all of the organs.

MARK F. BOYD

NEW PERIODICALS IN THE FIELD OF TROPICAL MEDICINE

Documenta Neerlandica et Indonesia de Morbis Tropicis (Quarterly Journal of Tropical Medicine and Hygiene). Ch. W. F. Winckel, Editor, Inst. Trop. Hygiene, 57 Mauritskade, Amsterdam O, Netherlands. Vol. 1, No. 1, March, 1949. Subscription per annum Hfl. 12, or \$4.50, or 22/6, sent to Riverenlaan 268, Amsterdam Z, The Netherlands.

Revista Brasileira de Malariologia. Publicada trimestralmente. Redactor-Secretario, Dra. Wanda C. Garcia, Serviço Nacional de Malaria, Rua Melo e Souza, 142, Rio de Janeiro, D. F., Brasil. Vol. 1, No. 1, Janiero, 1949. Gratis.

Zeitschrift für Tropenmedizin und Parasitologie. Herausgegeben von Dr. E. G. Nauck, Prof. Dr. E. Reichenow, Prof. Dr. H. Vogel, Bernard Nocht Institut für Schiffs- und Tropenkrankheiten, Hamburg 4, Germany. Four issues per annum, subscription per volume D. M. 58. Georg Thieme, Verlag, Diemershallenstrasse 47, Stuttgart-O, Germany.

The Chinese Review of Tropical Medicine. Editor-in-Chief, S. L. Hung. Vol. 1, No. 1, January 1948. Published by Institute of Tropical Medicine of the National University of Taiwan, Taipeh, Taiwan, China. Subscription not stated.

Bulletin of the Institute of Marine and Tropical Medicine of the Medical Academy in Gdańsk, Poland. J. Marzycki, Editor, Gdansk-Wrzeszcz, Poland. Quarterly, Vol. 1, No. 1, 1948. Subscription not stated.

Cinchona Review. Edited by Albert Hemming. Cinchona Products Institute, Inc., New York, Vol. 1, No. 1, June, 1949.

MEDICAL EDUCATION IN THE WESTERN PACIFIC^{1, 2}

MELVIN D. BIVENS, M.D.³

In 1944, long before the atomic age was ushered in by the historic bombing of Nagasaki, the Bureau of Medicine and Surgery of the U. S. Navy began to make plans (1) for the health of the people of the many Pacific Islands who were gradually being liberated from the Japanese. The plan envisioned the establishment of a school to train native medical and dental practitioners and native nurses to the end that medical teams would be available, following the training period, for all island populations. The island of Guam with the U. S. Naval and Guam Memorial Hospitals was chosen as the site for this school.

The curricula for the new schools were patterned after the leading medical, dental and nursing schools of the United States, and standard medical textbooks were to be used. A study was made of the British Central Medical School for Native Medical Practitioners at Suva, Fiji Islands (2). The experience accumulated there over a period of sixteen years offered numerous helpful suggestions which were incorporated into the basic plan.

Captain Richard H. Fletcher, MC, USNR, was the first Medical Officer in Command and the initial organization, requisitioning of equipment, laboratory supplies, etc., was completed before he left the U. S. for Guam in September, 1945 (3). He outlined the qualifications and method of screening prospective students and directed the work required to rehabilitate the facilities of the decommissioned Fleet Hospital #111 to school use.

Captain Fletcher speedily completed this work with the help of the Eighth and One Hundred Ninth NCB and the U. S. Naval School for Native Medical Practitioners and Native Nurses was commissioned at 1000, 2 January 1946. Later the School of Nursing was attached to Guam Memorial Hospital (4) and the School of Medical Practitioners' title was changed to School of Medical Assistants (5). The first class of medical assistants consisted of twenty-three students, six from Samoa, twelve from Guam, three from the Marshalls and two from Saipan.

This medical school was an entirely new venture for the Navy Department. A comprehensive Stanford-Binet test was given to all students entering the school. The results exhibited a wide range of scores which suggested operating two class groups. Ten students were started in the second semester work, eleven in the first semester and two were to study English and enter later. We have recently begun to use an intelligence test devised especially for the Pacific Island students by Mrs. Florence A. Vandam of the Trust Territory of the Pacific Is-

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² The opinions or assertions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

³ Formerly LTJG, MCR, USNR. Present address: Good Samaritan Hospital, 2266 N. W. Marshall St., Portland 10, Oregon.

lands Department of Education. It requires very little knowledge of English, but by methods of multiple choice in signs, pictures and phonograms, the alert students can be detected. A close correlation of the scores on this test with the students' progress in school has been noted.

Most of the students had been screened by medical officers in the dispensaries from the various islands according to qualifications set up by the commanding officer of the school. The applicant was to be of sound body and mind, of good moral character, single and between the ages of seventeen and twenty-four years. He was to possess a speaking and reading knowledge of English encompassing a minimum of 500 words (6). Age and marital status were waived in exceptional cases. Many of the students had served as health aides, interpreters and hospital attendants to the American Armed Forces. Five of the Samoans had attended the Central Medical School at Suva for one year, and several of the Guamanians had completed most of the requirements for high school graduation. In other words, the Commanding Officer chose the most intelligent and best educated candidates who were interested in studying medicine.

The biographies of some of the students as told in their English compositions are as interesting and intriguing as a fast moving adventure story. One was a teacher in a missionary school. He was twenty six years of age, his wife had been chosen for him by the missionary and they have four boys. Another was a native of the Gilbert Islands but was shipwrecked by a storm on the Marshall Islands during the war and was kept there by the Japanese. He later became an interpreter for the Medical Officer at Majuro and is now an outstanding student in the senior class.

A brief resumé of this Pacific Island civilization which jumped forward with a fifty year leap after almost 2000 years of slow nonaggressive progress will perhaps help understand better the mission of this school. Jeeps, bulldozers, airplanes, radios, hospitals and medicine are as fascinating and unique to many of these islanders as the two-wheel bull cart, tapicloth, lime pits, stone money and menstruation huts are to Navy dependents.

The area looking to the Navy for medical care includes American Samoa, Guam, and the islands of the Trust Territory. The Trust Territory of the Pacific Islands was the former Japanese Mandate and is made up of the Marshall Islands, the Eastern and Western Caroline Islands and the Northern Marianas Islands. All of this area consists of approximately two million square miles with a native population approaching 100,000 of Micronesian and Polynesian derivation. Approximately 125 islands are inhabited with populations ranging from three or four individuals, to as high as 25,000, excluding service personnel. Ten different languages are spoken but Japanese is the most widely understood (7).

The topography varies from volcanic islands with small mountains to coral atolls. Rainfall varies from semi-arid to precipitation of over 350 inches a year. Typhoons occur as often as five to six times per year in some areas.

Culture varies from that approaching Hawaiian standards to that of the most primitive in the world (7). Education depends upon whether schools have been conducted by missionaries or by the Japanese on the particular island in the

past. Two students had certificates stating they had five years of training as medical aides in a Japanese hospital on the Island of Jaluit. Interviews with these students showed, however, they they spent less than five hours per week in class. They had no laboratory work and no lectures after the first two years because of the war. They had no textbooks of their own but were allowed to read those of the doctors until they departed in 1944. After that they had two years of good experience suturing minor lacerations, delivering babies and treating minor ailments.

Many students possessed the minimum requirements for admission to the school (500 English words) and had only two or three years of elementary Japanese schooling. Due to the language difficulty of some of these it was deemed advisable to bring them to the school six months in advance of the class year for a period of intensive language training. A pre-school semester was then added to the four year curriculum. Many of these students possess keen minds and have proven themselves very capable in premedical and medical subjects after mastering a working knowledge of English.

Social practices vary greatly. In certain areas matriarchy of property and position is practiced (7). In these areas women occupy a high position in the community. Mothers choose husbands for their daughters and the young man comes to live with the girl's family. In other areas women are considered to be servants and laborers (7). Here they are greatly restricted in their actions and privileges. When a woman comes into the presence of a man on Yap she bends forward with her arm between her thighs and bows low until the man has passed.

Religion varies from one area 100 per cent Protestant to areas of 95 per cent Catholic. Other areas have nine different gods, all represented by different priests who are each responsible for one or two aspects of life (7).

Industry is confined to the production of copra and handicraft for exportation, and articles for home consumption such as mats, canoes and woven fabrics. Fishing for home consumption is widely practiced in some islands and to a very limited extent on others. Food consisting of breadfruit, taro, sweet potatoes, cocoanuts and chickens are produced on all islands. Breadfruit and taro are the chief articles of diet. The economic standards are not therefore based on products exported as most of the islands are self-sufficient, but upon products produced for local consumption (7).

At present, facilities for the care of the sick in the islands of the Trust Territory are provided by five main dispensaries and one subdispensary of from twenty five to eighty bed capacity located at the various civil administration units. Each of these units is staffed by two to three medical officers and three to twelve enlisted hospital corpsmen from the Navy. A group of native men and women who have received six months intensified training in sanitation and caring for the sick at these central dispensaries have been of great value in establishing numerous subdispensaries on the smaller islands. They act as first aid and sanitary personnel. Field trips by station ships are made at intervals of six weeks to all major inhabited islands. A medical officer holds sick call and a sanitary inspection. Sick who need hospitalization are brought back to the civil administration

unit dispensary. Patients requiring treatment beyond the scope of these dispensaries, such as cataracts, thoracoplasty, and operable carcinoma are transferred to Guam Memorial Hospital for treatment.

A central sanatorium of 200 bed capacity is being established on Saipan for care of patients with tuberculosis. A leprosarium is being established on Tinian for the care of the ninety known lepers. Fungus infections, yaws, tuberculosis, intestinal parasites, filariases, encephalitis and nutritional diseases are the leading pathological offenders. Venereal diseases are common in certain areas. Educational campaigns regarding the spread and methods of control of these diseases are being conducted among the natives by school teachers, health aides, and health survey groups. Movies have proven a valuable teaching aid, particularly the cartoon type.

It is believed that most of this work can and will be adequately handled by the medical assistants and nurses, under the supervision of a naval medical officer, when they have finished their training in the school here on Guam.

The allocation of students is made on the basis of population to be served, working toward an average of one medical practitioner for every 800 people in the islands of the Trust Territory. Guam now has 7 students and Samoa 14 (see Table I).

The allocation takes cognizance also of the desirability to develop medical teams by bringing to the school both medical assistants and nursing students from each island area.

The concepts incorporated in the basic plan for the school have been substantiated by the demonstration of the aptitude of students of the Pacific Island area to acquire scientific knowledge. The original idea that these students should not be removed too far from their own environment and customs to study medicine has been confirmed. The students are happy in school but have often expressed appreciation for their training so they will be able to help their people upon graduation. The cultural improvements obtained here will be reflected in the populations where they work.

The first class of ten students who are now seniors has for various reasons been reduced to five. One transferred to dental training, others dropped out voluntarily and some failed. These students have weathered the accelerated four and one-half year course and have proved their ability on the wards and clinics of the hospital. They also set the pattern for the classes that follow in student government, conduct and study habits.

The school has accepted twenty to twenty-five new students on July 1 every year for the beginning of pre-school training. The first semester of academic studies begins in January. Each semester consists of twenty-two weeks. A six-week Christmas vacation is granted each year, and all students who have been here twelve months are furnished transportation to their homes and back. A ten day rest period is also provided between semesters in June.

The student enrollment has increased to eighty one and the physical facilities have been gradually improved within the past twelve months. The laboratories all have concrete floors and Kemrock table tops with lead troughs and sinks.

Each desk is fully equipped with gas, water, electricity and compressed air. Chemical evacuation hoods, movie projector with sound equipment, bacteriological incubators, drying ovens, autoclaves and selected pathological slide sets are but a few of the items that make the school modern. A colony of guinea pigs have been raised for laboratory experiments and cages for dogs, monkeys and toads have been built.

TABLE I

ISLAND	CLASS					TOTAL	
	1949 June	1949 Dec.	1950 Dec.	1951 Dec.	1952 Dec.	Med. Assts.	Dental Assts.
Samoa	3	3 1*	5	3 1*	1*	14	3
Majuro	1		1	2	1 2*	9†	2
Kwajalein			3	1 2*	3 2*	7	4
Truk				2	5 2*	7	2
Ponape				2*	2 2*	2	4
Guam	1	2	4			7	
Saipan			2	1	1	4	
Koror & Palau				4 2*	1 2*	5	4
Yap & Ulithi				3	3	6	
Tinian			1			1	
Total.....						62	19

* Dental Assistants.

† Four post-graduate Japanese-trained practitioners.

For recreation we now have one baseball field, three volley ball courts, two tennis courts and a basketball court. Fishing and camping equipment has been obtained for weekend trips to Cocos Island where students fish, swim and relax on holidays and between semesters.

The new students arrive on Guam in July, usually by air transport, and each is assigned a private room with a steel locker, bed, desk, chair and lamp. A recreation hut equipped with a victrola, ping pong table and a large collection of

selected books is located between their Butler type barracks. Each student is provided with linens, laundry service and a scholarship fund of \$20.00 per month for clothing, toilet articles and other incidentals.

The first six months are spent in Pre-school Training (see Table II) except those enrolled with advanced standing. All students, excluding those on the honor roll, are required to study at least two hours (1900-2100) every evening

TABLE II
Pre-School training

	LECTURE HOURS PER WEEK
1. English:	
Basic English—Vocabulary and Grammar	5
Reading Exercises	5
Periodicals	2
2. Elementary Geography	5
3. Mathematics: addition, multiplication, subtraction and division of whole numbers. Introduction to fractions	5
Total.....	484

TABLE III
First semester subjects

SUBJECT	CONTENT	LECTURE HOURS PER WEEK
English:	Conversation, reading, grammar, spelling, public speaking	16
History:	Early European and American	6
Mathematics:	Arithmetic, algebra, geometry, trigonometry, high school level	11
Political Science:	Democracy and its evolution, current events	5
		—
		836

except Fridays and Saturdays, when they go to the movies, boxing matches or parties with the student nurses.

The students naturally tend to be clannish at first and segregate themselves with their fellow islanders who speak their language, but with the association in class, laboratory, softball, volley ball, boxing, tennis and basketball teams, they develop a school spirit and class competition. The school softball team beat the Guam All-Navy-Champions in 1947 and won the Medical Center championship in 1948.

The first semester introduces the student to history, higher mathematics and

political science. He still has sixteen lecture hours per week in English, which includes public speaking (see Table III).

The second semester introduces the students to the scientific world of biology, chemistry and physics for which they show a great deal of enthusiasm. Their English is continued through this semester to help them with composition and their scientific vocabulary (see Table IV).

If at the end of this semester the instructors feel that a student cannot keep up with the class or is not capable of attacking the heavier medical sciences, such as anatomy, bacteriology and physiology, he is placed in one of three categories: (a) He is placed on probation and must improve in his work with extra help; (b) Given opportunity to repeat a semester; (c) Dropped from school and

TABLE IV
Second semester subjects

SUBJECT	CONTENT	HOURS/WEEK	
		Lect.	Lab.
Biology:	Stressing material pertinent to physiology and anatomy. The single cell (plant and animals), hydra, arthropods, the frog, the human	4	6
Inorganic Chemistry:	Theoretical and applied fundamental principles. High school to college level	5	6
English:	Grammar 3 hrs. Reading 2 hrs. Periodicals 1 hr.	6	
Physics:	Electricity and mechanics, high school level. Heat, light, sound, stressing principles related to medical sciences. College level	5	6
Total		440	396

sent back to his home island. Some of those dropped for academic reasons have been trained as dental prosthetic technicians.

By this time the students have learned how to study. They have adjusted themselves to being away from their families and are capable of taking the big step into the field of medicine. As in many medical schools, we have some who are overwhelmed by the magnitude of anatomy and physiology. Strangely enough there are some who become so worried about the impossibility of learning all the facts that they burn the "mid-night oil" and their faces assume the normal expression of freshmen medical students anywhere.

The third semester brings them through 198 hours of gross anatomy, 220 hours each of physiology and bacteriology, 88 hours of histology, and 132 hours of organic chemistry (see Table V). At the end of this semester the dental students are transferred to the School of Dental Assistants but return for pharmacology.

They study in their fourth semester clinical laboratory methods, physiological

chemistry, parasitology, public health microbiology, and physical diagnosis which gives them a broad scientific background for their work on the wards in their junior and senior years (see Table VI).

TABLE V
Third semester subjects

SUBJECT	CONTENT	HOURS/WEEK	
		Lect.	Lab.
Anatomy:	General human. Including dog dissection, and embryology	3	6
Bacteriology:	General, stressing pre-clinical. College level	4	6
Histology:	General, college level	2	2
Physiology:	General, stressing principles pertinent to clinical medicine, college level	6	4
Organic Chemistry:	General—college	3	3
Total		396	462

TABLE VI
Fourth semester

SUBJECT	CONTENT	HOURS/WEEK	
		Lect.	Lab.
Clinical Laboratory Methods:	Medical school level	2	4
Physiological Chemistry:	Medical school level	6	3
Pathology:	General medical pathology, medical school level	6	6
Pharmacology:	Medical pharmacology and pharmacy in the laboratory	3	6
Physical Diagnosis:	Techniques of physical diagnosis, medical school level	3	
Total		440	418

The full time faculty consists of four medical officers, one warrant officer, two chief hospital corpsmen and four civil service employees with professional ratings. Two hospital corpsmen serve as laboratory assistants. The educational qualifications of these instructors are summarized below in Table VII.

The clinical teaching is organized under professors who are senior medical officers at the hospitals and are well qualified in their respective fields. They are

TABLE VII

POSITION	B.A.	B.S.	M.S.	M.D.	YEARS OF TEACH- ING EXPER.	YEARS OF LABORATORY EXPER.	OTHER REMARKS
Medical Officer (Commanding Officer)	x			x	13		
Medical Officer	x		Parasi- tology	x			
Medical Officer	x			x	5		1 yr. graduate work in zoology.
Medical Officer				x		2 years Pathol- ogy	
Warrant Officer Hospital Corps		x			4		5 yrs. experience as pharmacist.
Chief Hospital Corpsman	x				4		1½ yrs. of medical school. ½ yr. graduate work in education.
Chief Hospital Corpsman							1 yr. Pharmacy & Chemistry School— Navy. ½ yr. Physio- therapy School — Navy.
Civil Service (1)		x	Physiological Chemistry		1		2 yrs. clinical lab. work. 7 yrs. medical re- search Northwestern Univ. Medical School & Navy.
Civil Service (2)		x			3	3	2 yrs. graduate work in a Med. Tech. School. 1 yr. Mayo Clinic Chem.
Civil Service (3)	x				1		
Civil Service (4)	x				2		

responsible for organizing the lecture courses and supervising the bedside teaching as well. Other medical officers are selected by them to help carry out these duties.

The fifth and sixth semester curriculum is given in the following Table VIII.

The seventh and eighth semesters' clinical work is supplemented by field trips to the dispensaries in the outlying districts of Guam, the water purification stations, sewage disposal plants and public restaurants. They also attend the regular hospital staff conferences, tumor clinic, and public health meetings. The appreciation and challenge of the history of medicine and medical ethics has been included in their curriculum as well as a course in Medical Administration (see Table IX).

A well equipped medical library supplies the students with a wide selection of the medical periodicals. Short theses are required of the senior students at the

TABLE VIII
Fifth and sixth semesters

SUBJECT	LECTURE HOURS
Medicine	
Internal Medicine.....	176
Pediatrics.....	88
Neurology and Psychiatry.....	66
Diseases of the Chest.....	88
Dermatology and Syphilology.....	88
Public Health and Preventive Medicine.....	44
Cardiology.....	44
Surgery	
General.....	220
Orthopedics.....	44
Anesthesia.....	44
Obstetrics and Gynecology.....	88
Clinical Work on Hospital Wards.....	880
Clinical Conference.....	88
Library.....	44
Total.....	2002

close of each clinical service as they rotate through the hospital. They spend 792 hours in the clinics and general dispensary, 88 hours in planned clinical conferences and a minimum of 220 hours in collateral reading in the library.

Our students interview and examine an abundance of clinical patients. During the past year (September 1947 to September 1948) Guam Memorial Hospital had a total of 2,739 admissions, 220 surgical operations and 301 births. There were 10,780 patients treated in their out-patient departments and our junior and senior students work under the supervision of the medical officer in all of the wards and clinics. Major surgical cases from all of the islands of the Trust Territory are sent here. Obstetrical cases, parasitic and fungus infections, tuberculosis, accidental injuries, gastrointestinal infections and infestations, encephalitis and acute communicable diseases are seen in abundance. Most of the above list

are common to the areas in which the students will later practice. Training in these endemic diseases as provided by Guam Memorial and U. S. Naval Hospital and the Public Health Department is quite possibly more intensive than would be the case in stateside hospital training.

In October 1948 the medical staffs of the Guam Memorial and U. S. Naval Hospitals were combined under a single Chief of Medicine, Chief of Surgery, etc. The students now get the advantage of working in the Eye, Ear, Nose and

TABLE IX
Seventh and eighth semesters

SUBJECT	LECTURE HOURS
Medicine	
Nutrition.....	22
History of Medicine and Medical Ethics.....	22
Radiology.....	44
Medical Administration.....	22
Therapeutics.....	88
Toxicology.....	22
Physical Medicine.....	22
Pharmacy.....	22 44 Lab. Hrs.
Surgery	
Minor and Applied Surgery.....	44
Urology.....	22
Orthopedics.....	44
Sanitation.....	32
Obstetrics.....	22
Parasitology.....	44 44 Lab. Hrs.
Clinical Clerkships.....	792
Including General Dispensary, Prenatal, EENT, Dermatol- ogy, Surgery, Dentistry, Orthopedics, Tuberculosis, Fluoros- copy, Gynecology, Yaws, Main Laboratory and Postmortem Examinations.	
Clinical Conferences.....	88
Medical Library.....	220
Total.....	1572 88 Lab. Hrs.

Throat, Dermatology, Orthopedic, Urologic and Medical Clinics of the U. S. Naval Hospital which treated 12,313 out-patients during the past six months. Beginning in January 1949, they will also work in the U. S. Naval Hospital wards and surgeries which totaled 6,975 admissions last year. Our students attended most of the 265 autopsies performed during the past 12 months.

Recently a new problem was encountered in the school. Four Japanese-trained Marshallese practitioners were sent here for a one year refresher course. These men range from 25 to 43 years of age. All of them attended Japanese training schools in the hospitals on the island of Jaluit for a period of five years. Some of

these practitioners have been practicing among their people for twenty five years, and according to reports from the Naval Medical Officers, they have done a good job. They have gained the respect of the people and fill a definite need in the public health organization. Since 1946 all of these men have spent from six to fourteen months in the U. S. Naval Dispensaries in training and review and should profit from refresher training.

The senior student from Majuro in the Marshall Islands is a part-time instructor for these men. His efforts are complemented by the extra teaching and cooperation of part of the full time faculty; these men received a lot of individual instruction. The youngest man in this group understands English well and explains the subject matter to the others in Marshallese. Their progress has been rapid, particularly in the problems of laboratory methods, pathology and bacteriology encountered in the patients they examine.

I have worked closely with our junior and senior students on the wards and clinics. I have observed them in Obstetrics, Medicine, Surgery and Admission Room. They daily demonstrate their ability for close observation, make excellent histories and physical examinations, and accept their responsibilities well.

We believe that the mission of the school is being fulfilled and that these students will play a great part in the growing civilization of these strategically located islands of the Pacific. After twelve months interne training which is planned for them, they will be able to assume much of the work of the medical and public health departments of the Naval Government and release some of the military personnel for duty elsewhere. I am sure they will prove themselves valuable assets to their people and the Navy.

The goal for the number of graduate medical assistants is seventy seven (Trust Territory 56, Samoa 14, Guam 7) by December 1954. It has been estimated that this number will be sufficient to fill the needs at that time. The high schools of the islands will be graduating sufficient students by 1954 so that any further need for medical men can be filled by selecting the best graduates to attend standard colleges and medical schools.

Graduates of the School of Medical Assistants, as well as those completing the refresher course, will appear before a board of examiners, and if found qualified will be granted a license to practice.

It is anticipated that upon being licensed, medical assistants will return to their home district and will be employed as public health medical assistants either on their home islands or at the Civil Administration Unit Dispensary for a period of two years. After the two year period, these medical assistants may either be retained as public health officers or be allowed to enter private practice on any island of their choice within the district, or be employed as part-time public health officers with permission to engage in private practice.

While they are engaged as public health personnel, it is tentatively planned that all medical equipment and supplies will be furnished by Trust Territory or Naval Government, with housing supplied by the local government. Upon entering private practice, expenses incident to obtaining supplies and equipment

should be borne by the individual with necessary financial assistance through loans from the Government of the Trust Territory.

The extent of the practice conducted by the individual will be limited to general practice, general medicine, obstetrics and minor surgery. The Naval medical officer from the Civil Administration Unit will, through periodic field trips and inspections, supervise the practice of the medical assistants on the various individual islands (8).

The accomplishments of this school can best be evaluated ten or fifteen years from now by visiting the Pacific Islands and observing the medical assistants' work. It would be interesting indeed to stop off in Samoa or Yap in 1960 and check on the achievements of our graduate medical assistants.

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EXPERIMENTAL INFECTION OF THE RABBIT WITH *ENDAMOEBIA HISTOLYTICA*

JOHN E. TOBIE¹

INTRODUCTION

In the past, studies on the immunology and chemotherapy of amoebiasis have been hampered by the lack of a suitable experimental animal. Monkeys, dogs, kittens and rats have been employed but found to be rather unsatisfactory because of one or more of the following reasons: (a) the expense of the animal; (b) the difficulty encountered in handling the animal; (c) the difficulty of producing an infection in the animal; (d) the duration of the infection; and (e) the pathological changes produced.

Several investigators have produced amoebic infections in the rabbit. They were successful in only a limited number of animals and the pathological changes were minimal. Huber (1) after repeated feedings of cysts from human stools to 8 different rabbits, produced infections in 4 of these animals. The lesions were small and confined to the caecum. Deep or extensive ulceration was not observed. In 1926 Thomson (2) inoculated numerous rabbits by feeding cysts of *Endamoeba histolytica* and by inoculating trophozoites intrarectally. One rabbit, which had been fed the organism, developed caecal amoebiasis. Examination of the caecum revealed one small area of inflammation and ulceration. Westphal (3) attempted to produce amoebic infections in rabbits by the intragastric administration of cysts and the intracaecal inoculation of trophozoites. After numerous experiments, one rabbit contracted the infection. The small lesions that were produced were confined to the appendix. Due to the limited success of previous workers, it was felt that more extensive experiments should be conducted on rabbits in an effort to obtain a higher percentage of infections and to produce lesions more closely resembling those found in the human.

MATERIALS AND METHODS

Two series of rabbits were inoculated with *Endamoeba histolytica*. Within a period of 2 months, 33 rabbits (Experiment I) were inoculated intraoesophageally with cultivated cysts and within a period of 11 days, 11 rabbits (Experiment II) were inoculated intracaecally with trophozoites.

The regimen and inoculation of the various animals of Experiment I was according to the following procedure. Twelve- to thirteen-week-old rabbits, of both sexes, belonging to the New Zealand White and Dutch strains were utilized in the experiments. One week prior to inoculation each rabbit was placed exclusively on a grain-bread diet, similar to the diet used by Westphal (3). The grain consisted of a mixture of cracked corn, oats and bran, while the bread was of the whole-wheat variety. The animals were kept on this diet throughout the entire

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experiment. All of the rabbits of this series were inoculated intraoesophageally by means of a 10-ml. syringe fitted with a $1\frac{1}{4}$ inch 18-gauge needle. The point of the needle was cut off and a smooth, solder tip connected to its end. This bulbous tip prevented injury to the animal during the inoculation procedure. An attendant held the rabbit's mouth partially open while the blunt needle was introduced through the side of the mouth and into the proximal portion of the esophagus. Approximately 1 ml. of a concentrated inoculum, containing numerous cysts of *E. histolytica*, was then forced from a syringe into the esophagus. It was found that the animals could be inoculated in this manner very rapidly and efficiently. The method was found to be more practicable than intragastric inoculation by means of a stomach tube.

Eleven rabbits (2 males and 9 females) belonging to the New Zealand White strain were utilized in Experiment II. Six of the rabbits were inoculated at the age of 8 weeks and the remaining 5 at the age of 9 weeks. At the time of inoculation the weights of the individual rabbits varied from 1137 to 2131 grams. They were placed on the grain-bread diet, previously described, for a period of 2 to 3 weeks prior to inoculation and were kept on this diet throughout the entire experiment. The animals were inoculated intracaecally in the following manner. Each rabbit was anesthetized with nembutal which was administered intravenously. A 2- to 2.5-cm. skin incision was made in the right lower quadrant of the abdomen, the muscles separated with a probe, and an opening made into the abdominal cavity. Retractors were then inserted into the incision and the caecum located. By means of a 5-ml. syringe fitted with a 20-gauge needle, trophozoites of *E. histolytica* from 48-hour cultures were introduced into the caecum. The abdomen was then closed with 3 skin clips. It was found unnecessary to suture the separated muscles. Sterile precautions were taken throughout the operative procedure. Each of the first 6 animals in this series was inoculated with the harvest (approximately 2 ml.) from 5 cultures and each of the remaining 5 animals with the harvest (approximately 0.5 ml.) from 1 culture. The amount of inoculum was reduced in the latter animals because the incubation period was too short and the infections too acute in the first group.

One strain of *E. histolytica* (Strain 200) was used for both series of rabbit inoculations. It was obtained at sigmoidoscopy from a patient suffering from amoebic dysentery, transferred to a dog and subsequently isolated in culture. A mixed bacterial flora was associated with the amoebae. At the time of the inoculations in Experiment I, the amoebae had been under continuous cultivation for a period of 10 months. For this experiment cysts were produced from the cultivated trophozoites by growing the amoebae in Locke-egg medium for 6 transfers in the absence of rice starch, then for 1 transfer in the presence of starch. The cultures were pooled and the cysts concentrated by centrifugalization. In Experiment II the same strain of *E. histolytica* was used but at the end of the 10-month period of cultivation it was passed through a rabbit, put back into culture, and kept under continuous cultivation for an additional period of 7 months. For this experiment only, the solid material (containing rice starch, bacteria and tropho-

zoites) was aspirated from the bottom of the culture slant and inoculated directly into the caecum of the rabbit.

Prior to the inoculations in Experiments I and II, fecal examinations performed on all rabbits, by the use of the direct smear method and the zinc sulphate centrifugal flotation technique of Tobie (4), failed to reveal any natural amoebic infections.

RESULTS

Of the 33 rabbits in Experiment I, inoculated intraoesophageally with cultivated cysts of *E. histolytica*, 6, or 18 per cent, contracted intestinal amoebiasis as demonstrated by the recovery of amoebae from intestinal scrapings and the production of lesions. Trophozoites of *E. histolytica* were detected in dysenteric material taken from the anus of one rabbit (No. 54.8) 15 days after inoculation. In the other 5 infected animals the diagnosis was established at the *post mortem* examination. Two of the infected rabbits (Nos. 51.1 and 51.2) died 14 days after inoculation and the remaining 4 (Nos. 51.3, 51.7, 54.2 and 54.8) were sacrificed 15–21 days after inoculation.

Of the 11 rabbits in Experiment II, inoculated intracaecally with trophozoites of *E. histolytica*, 10, or 91 per cent contracted intestinal amoebiasis as demonstrated in the above manner. Eight of the infected rabbits were diagnosed *ante mortem* or before they were sacrificed by the demonstration of amoebae in mucus scraped from the anus or aspirated from the rectum. Diarrhea in the 8 rabbits began 5 to 9 days after inoculation. In the other two infected animals the diagnosis was established at the *post mortem* examination. Four of the infected rabbits (Nos. 57.1, 57.2, 57.7 and 57.11) died 5–12 days after inoculation and the remaining 6 (Nos. 57.3, 57.4, 57.5, 57.6, 57.8 and 57.9) were sacrificed 6–13 days after inoculation.

At necropsy of 1 of the rabbits in Experiment I and 8 of the rabbits in Experiment II, fecal material was placed in Locke-egg medium and subsequent transfers revealed typical trophozoites of *E. histolytica*. The history of this particular strain from Experiment II is thus: human → dog → culture → rabbit → culture → rabbit → culture.

Many of the trophozoites of *E. histolytica* from the animals of Experiments I and II had numerous bacteria within the endoplasm. An amoeba from one rabbit had ingested a spore of *Geotrichum* sp., a fungus which was found in the intestinal contents of most of the animals used in the present study. Many other amoebae contained a protozoan flagellate within their cytoplasm, while others had ingested as many as 20 erythrocytes. Cysts were not observed in any of the 16 infected rabbits.

In the caecum and appendix of most of the infected rabbits there was very little or no particulate fecal material but rather an abundance of mucus. Many of the animals which had lesions only in the caecum and appendix, had fecal pellets in the colon, while in those animals in which the colon was involved, there were no fecal pellets. It was surprising to note that even in the fulminating cases

a relatively small amount of macroscopic blood could be demonstrated. However, blood was observed microscopically.

MACROSCOPIC PATHOLOGY

The following description of the macroscopic pathology is based upon the 16 infected rabbits of Experiments I and II. Both the small and large intestines were removed at necropsy. Before the intestines were opened, cream-colored lesions were observed through the normally thin caecal wall and the thicker appendiceal wall (Figures 1 and 2). The lesions, when observed from the mucosal surface, were covered with a cream-colored to yellow exudate. In the individual rabbit the number of lesions varied from a few to hundreds and the size from



FIG. 1. LESIONS IN THE APPENDIX OF A RABBIT (54.8)

0.5 mm. to 50 mm. (Tables 1 and 2). A definite pattern of the distribution of the lesions was evident in the caecum and appendix.

In the less acute infections the lesions were confined to the area of the caecum adjacent to the sacculus rotundus or to this area and the appendix. In general, these lesions were of the small, round type and varied in diameter from 0.5 mm. to 3 mm. The lesions of the appendix tended to be more concentrated in the base and in the distal third of the organ (Figure 3). The openings of the lesions in the terminal portion were somewhat irregular in outline in contrast to the round appearance of the same lesions when seen from the serosal aspect.

Those rabbits having more acute infections had lesions not only in the area of the caecum adjacent to the sacculus rotundus and the appendix, but also scattered throughout the rest of the caecum. In several of the animals the small,

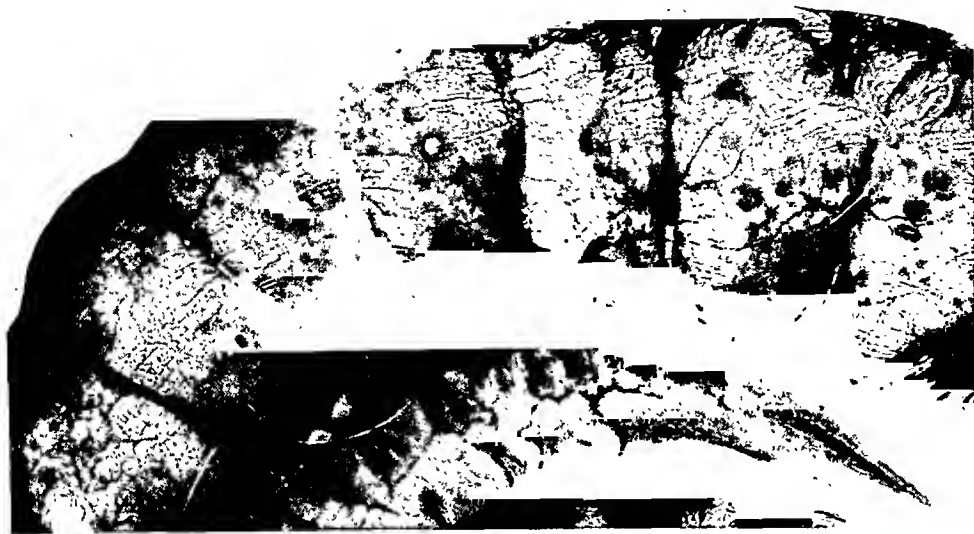


FIG. 2. LARGE LESION AND SEVERAL SMALL LESIONS IN THE MEDIAN PORTION OF THE CAECUM OF A RABBIT (54.8)

TABLE 1

Distribution of lesions in rabbits inoculated intracaecally with trophozoites of E. histolytica (Experiment II)

RABBIT NO.	CAECUM (AREA ADJACENT TO SACCULUS ROTUNDUS)		CAECUM (EX-CLUSIVE OF AREA ADJACENT TO SACCULUS ROTUNDUS)		APPENDIX		TERMINAL ILEUM		COLON		LENGTH OF EX-PERIMENT IN DAYS
	Lesions		Lesions		Lesions		Lesions		Lesions		
	No.	Size	No.	Size	No.	Size	No.	Size	No.	Size	
		mm.		mm.		mm.		mm.		mm.	
57.1	1	15	1	7	103	0.5-1	0	0	0	0	6
57.2	150	1-2	1	3	75	0.5-1	0	0	0	0	7
57.3	50	1-2	200	1-10	160	0.5-1	28	0.5-1	300	0.5-5	10
57.4	50	1-2	1	50	39	0.5-1	0	0	0	0	6
57.5	60	1-2	0	0	44	1	0	0	0	0	7
57.6	48	0.5-1	2	1	12	1	1	1	0	0	9
57.7	1	25	183	0.5-20	92	0.5-1	42	0.5-1	250	0.5-4	12
57.8	62	0.5-1	0	0	0	0	0	0	0	0	6
57.9	43	0.5-8	126	1-25	111	0.5-2	22	0.5	26	0.5-7	13
57.10	0	0	0	0	0	0	0	0	0	0	11
57.11	1	17	40	1-12	128	0.5-1	32	0.5-1	250	1-4	9

round lesions, in the area of the caecum adjacent to the sacculus rotundus, had coalesced to form one confluent lesion. The smaller caecal lesions, measuring 1 mm. to 5 mm., were generally round while the larger lesions, measuring 5 mm.

to 50 mm., were ordinarily more irregular in shape. One large lesion, measuring 26 mm. by 10 mm., was situated in the median portion of the caecum of rabbit No. 54.8, and communicated with the intestinal lumen by three large openings (Figure 4). Apparently this lesion was the result of the undermining and coalescing of three smaller ones. One of the most striking features of the large caecal lesions was their tremendous thickness. The caecal wall, in the area of the lesions, was often 2 to 4 times as thick as the normal caecum. In certain areas the caecal

TABLE 2

Distribution of lesions, and ranges in numbers and sizes of lesions in 10 rabbits infected with E. histolytica (Experiment II)

	CAECUM (AREA ADJACENT TO SACCULUS ROTUNDUS)	CAECUM (EXCLUSIVE OF AREA ADJACENT TO SACCULUS ROTUNDUS)	APPENDIX	TERMINAL ILEUM	COLON
Percentage of infected animals having lesions	100%	80%	90%	50%	40%
Range in numbers of lesions.....	1-150	1-200	12-160	22-42	26-300
Range in size of lesions (mm.).....	0.5-25	1-50	0.5-2	0.5-1	0.5-7

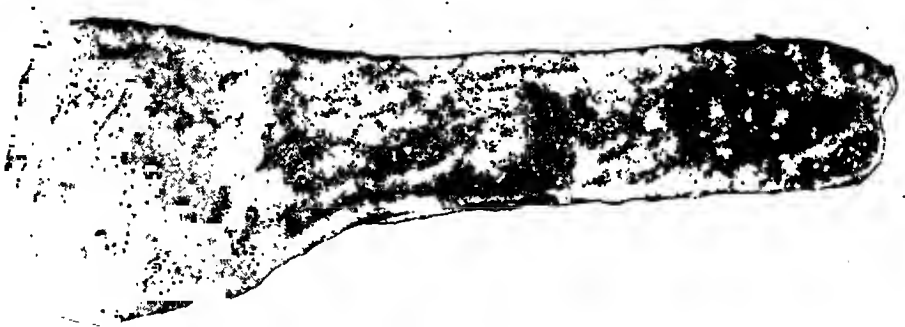


FIG. 3. LESIONS IN THE APPENDIX OF A RABBIT (54.8), AS SEEN FROM THE MUCOSAL SURFACE

mucosa was somewhat hyperemic and many of the blood vessels were injected. Small petechial haemorrhages were observed in the mucosa, particularly in the folds of the spiral valve. In the more acute infections of Experiment II, large haemorrhagic patches were seen in the caecum when viewed from the serosal surface. The number of lesions in the caecum varied from 1 to 200.

The animals having the most acute infections had numerous lesions distributed throughout the caecum, appendix, terminal ileum and colon. One rabbit (No. 54.8) also had small lesions, measuring 1 mm. to 2 mm., in the duodenum and jejunum. A few of the lesions of the colon were round but most of them were quite irregular in shape. They varied in size from 0.5 mm. to 7 mm. In the as-

ending colon the lesions tended to be more numerous in the haustra where there was stasis of the fecal contents. No lesions or very few were detected in the transverse colon. The lesions of the descending colon and rectum were often very numerous. Confluent lesions were observed in certain cases. In the 5 animals of Experiment II, which had involvement of the terminal ileum, the lesions were confined to the Peyer's patch which is located approximately 4 inches from the sacculus rotundus. They were of the small, round type, measuring 0.5 mm. to 1 mm. in diameter, and varied in number from 1 to 32.

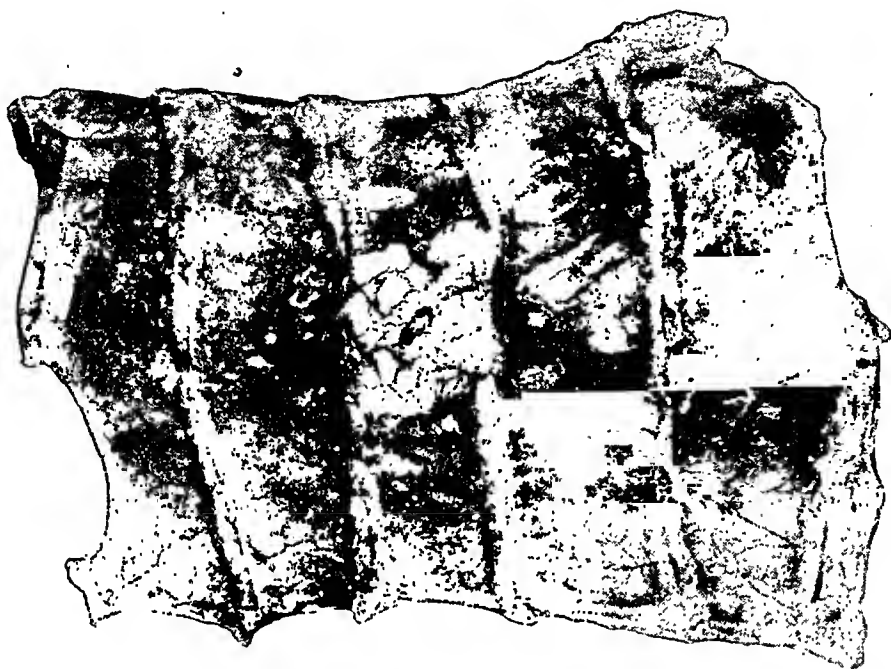


FIG. 4. THE LARGE LESION OF FIGURE 2 AS SEEN FROM THE MUCOSAL SURFACE

MICROSCOPIC PATHOLOGY

Since this study was not planned as one in histopathology, tissues of only 2 rabbits (Nos. 51.7 and 54.8) were examined microscopically. Lesions of the intestine varied in severity from those involving the mucosa alone, in which only small areas of tissue destruction had taken place, to those with extensive involvement of both the mucosa and submucosa. The small superficial lesions in the caecum were noted particularly in the folds of the spiral valve. The lesions in the appendix, which appeared round macroscopically, were usually bottle-shaped microscopically. These "bottle ulcers" were striking and frequently confluent in the appendix (Figure 7). In the large lesions of the caecum, there was considerable thickening of the submucosa and ragged, overhanging mucosa formed the edges of the lesions (Figure 5). The inflammatory reaction associated with the large lesions extended to and focally involved the muscularis. Figure 8 illustrates one of the lesions observed in the small intestine demonstrating the usual



FIG. 5. CROSS SECTION OF LARGE CAECAL LESION OF FIGURE 2

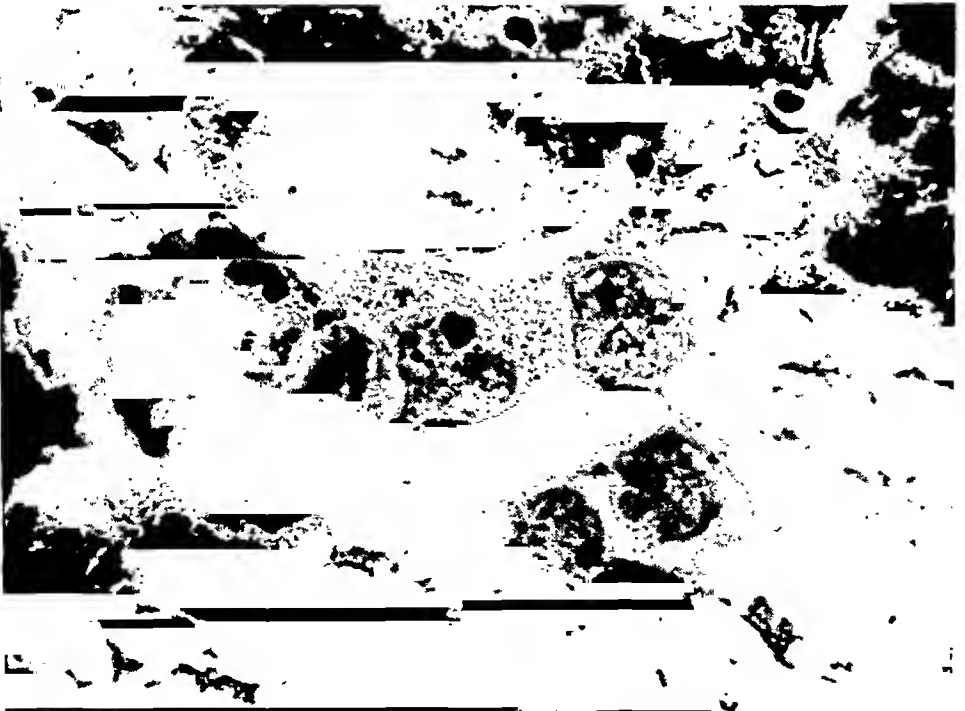


FIG. 6. TROPHOZOITES OF *Endamoeba histolytica* IN NECROTIC SUBMUCOSAL TISSUE

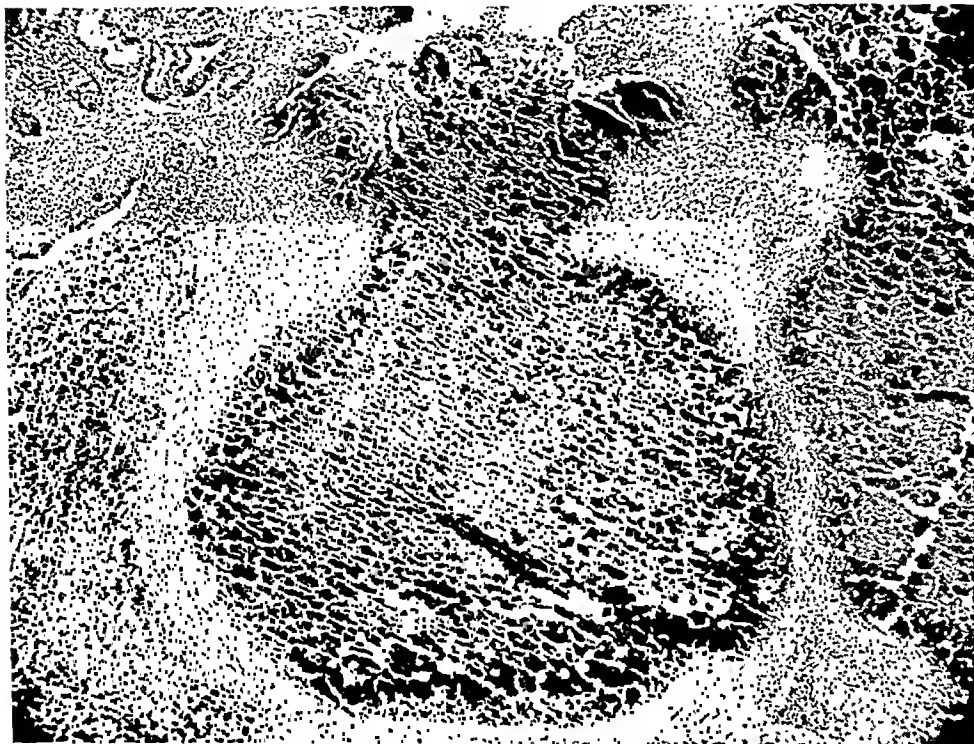


FIG. 7. "BOTTLE" ULCER IN APPENDIX OF A RABBIT (54.8)



FIG. 8. LESION IN SMALL INTESTINE OF A RABBIT (54.8)

necrotic processes which prevail in an amoebic lesion. Trophozoites of *E. histolytica* (Figure 6) were seen in all lesions and were situated principally at the edges of the necrotic material near the more normal mucosal and submucosal tissues. The amoebae were often quite numerous and some contained erythrocytes. It should be noted that, in certain respects, the microscopic pathology resembled that seen in the human.

DISCUSSION

The amoebic lesions produced in the present study were quite similar grossly to those seen in the human, even more so than those ordinarily produced in the dog. Tobie (5) observed in the experimental infection of dogs that the lesions often involve only the mucosa. The muscularis mucosae in the dog apparently offers considerable resistance to the penetration of the amoebae. In the present experiments it appeared as if the muscularis mucosae of the rabbit offered very little resistance to the amoebae because numerous lesions involving the submucosa were produced as early as 5 days after inoculation. Furthermore, the amoebae produced considerable thickening of the submucosa as was seen in the large flask-shaped lesions. The lesions in these rabbits were more numerous and extensive than those experimentally produced in rats by Jones (6). It also seems likely that in the present series of amoebic infections the rabbits would not have recovered spontaneously, as is so often the case in experimental amoebiasis in rats. The short course of infection and the nature of the pathological changes in the rat tends to make this animal rather undesirable for critical drug-testing experiments.

Quite a definite pattern of the distribution of lesions was observed. It appeared as if the amoebae had first attacked the lymphoid tissue of the area of the caecum adjacent to the sacculus rotundus and that of the appendix. After this initial invasion, lesions were then produced in other portions of the caecum and terminal ileum. Finally, in the most acute infections, there was involvement of the colon. In man, the lesions occasionally found in the small intestine are ordinarily confined to the terminal ileum. However, one rabbit (No. 54.8) also had lesions throughout the duodenum and jejunum, as well as the ileum. It is speculative as to whether the amoebic cysts had excysted in the duodenum or jejunum and produced lesions in these locations or whether the amoebae had excysted in the ileum and migrated back into the duodenum and jejunum as the infection became more acute.

The severity of the infections varied from those with a few small lesions to those with numerous small and large lesions. Many of the latter were fulminating cases and the amoebic infection did or probably would have produced the death of the animal. In general, the infections in Experiment I were less severe than those in Experiment II. Likewise, the percentage of "takes" was 18 per cent as compared with 91 per cent respectively. This might be expected since the rabbits of Experiment I were inoculated intraoesophageally with cysts and those of Experiment II were inoculated intracaecally with trophozoites. In the first experiment it seems likely that in certain animals the cysts did not survive pas-

sage through the stomach or did not excyst and survive in the terminal ileum or caecum. The method of inoculation in Experiment II also probably explains the shorter prepatent period.

The rabbits employed in Experiment I were 12-13 weeks old while those of Experiment II were 8-9 weeks old and all were kept on a grain-bread diet. Even though a higher percentage of "takes" was obtained in the somewhat younger rabbits, it is felt that the method of inoculation rather than the age of the animal was responsible for this difference. Further experiments will have to be conducted to determine the influence of age and diet on the experimental production of amoebic lesions. It has been established that in dogs the diet plays a most important role in the production of lesions and the maintenance of the infection.

It has often been stated in textbooks that trophozoites of *E. histolytica* from freshly passed stools or from scrapings of lesions, do not contain bacteria. In the present experiments this did not hold, because many amoebae taken from scrapings of lesions were observed to contain numerous bacteria, protozoan flagellates and even a fungus. It would appear that in the rabbit, *E. histolytica* avidly ingests not only tissue juices but also bacteria and other organisms found in the intestinal contents.

In comparison with the lesions produced heretofore in the various laboratory animals, the lesions in the present rabbits were of a much more extensive nature. Because of the large amount of tissue damage, it seems quite possible that the rabbit may prove to be a good experimental animal for immunologic and chemo-therapeutic studies in amoebiasis. ✓

SUMMARY

1. Thirty-three rabbits (Experiment I) were inoculated intraoesophageally with cultivated cysts of *Endamoeba histolytica*. Of this group of animals, 6, or 18 per cent, contracted intestinal amoebiasis as was demonstrated by the recovery of amoebae from intestinal scrapings and the production of lesions.

2. Eleven rabbits (Experiment II) were inoculated intracaecally with trophozoites of *E. histolytica*. Of this group of animals, 10, or 91 per cent, contracted intestinal amoebiasis as was demonstrated by the recovery of amoebae from intestinal scrapings and the production of lesions.

3. Flask-shaped lesions and "bottle" ulcers were produced in the various animals.

4. In one rabbit lesions were observed not only in the large intestine but also in all portions of the small intestine.

5. The severity of the infections varied from those rabbits having a few small lesions to those having numerous small and large lesions. Many of the latter were fulminating cases and the amoebic infection often produced the death of the animal.

6. Many of the trophozoites of *E. histolytica*, taken from scrapings of lesions, contained not only erythrocytes, but also numerous bacteria, protozoan flagellates and a fungus.

7. It seems quite possible that the rabbit may prove to be a most satisfactory

experimental animal for immunologic and chemotherapeutic studies in amoebiasis.

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STUDIES OF THE INFLUENCE OF OXYQUINOLINE DRUGS ON GROWTH OF *ENDAMOEBA HISTOLYTICA* AS MEASURED IN BLOOD IODINE LEVELS OF MAN^{1, 3, 4, 5}

ALVA A. KNIGHT,² DONALD W. TARUN, AND JEANNE MILLER

A study by Knight and Miller (1) of the levels of blood iodine obtained when the oxyquinoline drugs, Anayodin, Chiniofon, Diodoquin, and Vioform were given to man in recommended therapeutic doses, revealed a level of approximately 100 gammas in Anayodin and Chiniofon, 400 gammas in Vioform, and 800 gammas in Diodoquin. It further revealed the absorbability as measured by iodine intake and percentage recovery in the blood to be greatest in Vioform, next in Diodoquin, and least in Anayodin and Chiniofon.

This study has been extended in an attempt to determine the level of the drugs, as measured by blood iodine, necessary to inhibit the growth of *Endamoeba histolytica* in vitro. A liquid media was needed in which *Endamoeba histolytica* and its accompanying bacteria would grow and which could be agitated to give uniformity each time before withdrawing samples for determination of the growth of *Endamoeba histolytica* and bacteria. Equal parts of serum and egg infusion proved satisfactory.

We originally did counts at 12, 24, 48, and 72 hours on both *Endamoeba histolytica* and bacteria. The peak of the *Endamoeba histolytica* growth curve was found to be between 48 and 72 hours. The bacterial count remained high throughout the 72 hours. Forty-eight hours was accepted as an adequate period of incubation inasmuch as optimum amoebic growth in the control cultures occurred at this time.

The iodine content of serum was found to run slightly higher than the previously determined iodine levels in whole blood.

By greater effort to prevent diarrhea, it was found higher levels of serum iodine could be obtained.

It appears that the serum iodine level is inversely proportional to the growth of *Endamoeba histolytica*. This holds true regardless of which of the oxyquinolines is used.

All media iodine levels were one-half that of the serum levels since they consisted of equal parts of egg infusion and serum.

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⁴ Acknowledgement is made to Dr. William H. Welker of the Department of Biochemistry of the University of Illinois College of Medicine for the use of laboratory facilities.

⁵ Read before the annual meeting of the American Society of Tropical Medicine, New Orleans, Louisiana, December 5-8, 1948.

During the latter part of our study, fecal iodine levels in Diodoquin and Vioform were run on several patients after treatment and were found to be high.

Studies on the levels of blood and fecal iodine obtained from potassium iodide by mouth and sodium iodide by vein together with the inhibitory effect on *Endamoeba histolytica* growth *in vitro* will be reported later.

METHODS

Amoebacidal and bactericidal activity of the drugs used was determined *in vitro* as follows: The test organism was the NRS strain of *Endamoeba histolytica*,

TABLE I
Diodoquin

PATIENT	IODINE* IN CULTURE AFTER TREATMENT	AMOEBAE† PER MM. ³ AT 0 HOURS	AMOEBAE PER MM. ³ AT 48 HOURS			BACTERIA† × 10 ³ PER MM. ³ AT 0 HOURS	BACTERIA × 10 ³ PER MM. ³ AT 48 HOURS		
			Control	Before treatment	After treatment		Control	Before treatment	After treatment
W. C.	185	17	125	125	1	23	470	385	399
F. S.	472	12	111	132	0	19	275	273	259
R. D.	325	14	121	133	2	35	510	480	510
M. F. S.	289	11	109	117	0	24	318	292	296
C. W.	268	19	93	69	2	18	400	362	410
C. R. L.	409	19	93	91	0	18	400	410	371
M. F. S.	638	16	82	95	0	17	380	430	409
H. B.	214	13	63	75	0	29	470	510	500
J. M.	338	13	63	68	0	21	470	510	420
J. C.	562	11	61	79	0	21	520	390	480
D. C.	156	8	77	81	0	12	580	460	470
A. C.	409	10	84	30	0	17	430	590	530
L. B.	508	10	84	101	0	17	430	470	450
M. E.	461	16	92	79	0	28	440	590	440
M. M. E.	447	10	75	73	0	23	590	610	500
Averages.....	380	13	89	90	1	21	440	445	430

* In gammas per 100 ml. media is 1:1 serum-egg infusion with rice starch.

† Calculated from count of inoculum.

obtained through the courtesy of Dr. William Balamuth of Northwestern University. The accompanying bacterial flora consisted of *Escherichia coli* and two enteric gram-negative rods, not further identified. Stock cultures were maintained in the egg infusion media of Balamuth and Sandza (2). Pooled sediment from 48 hour cultures was used for inoculum at the time a test run was made. There were transferred to each of three tubes containing 5 ml. of 1 part serum and 1 part egg infusion 0.2 ml. of inoculum of known amoebic and bacterial concentration. A small loop of sterile rice starch was added to each tube. Two tubes served as controls, one containing serum from a normal individual without amoebiasis and undergoing no treatment and the second containing serum from a patient with amoebiasis before treatment. The third tube contained serum

from the same patient after treatment. The tubes were incubated at 37°C. for 48 hours and at this time amoeba counts were made and bacterial counts set up. Amoebae were counted by the method of Paulson (3) and a minimum of four counts was made for each culture. Bacterial counts were made by the saline decimal dilution method and 0.1 ml. samples of the terminal dilutions were

COMPARISON OF AVERAGES

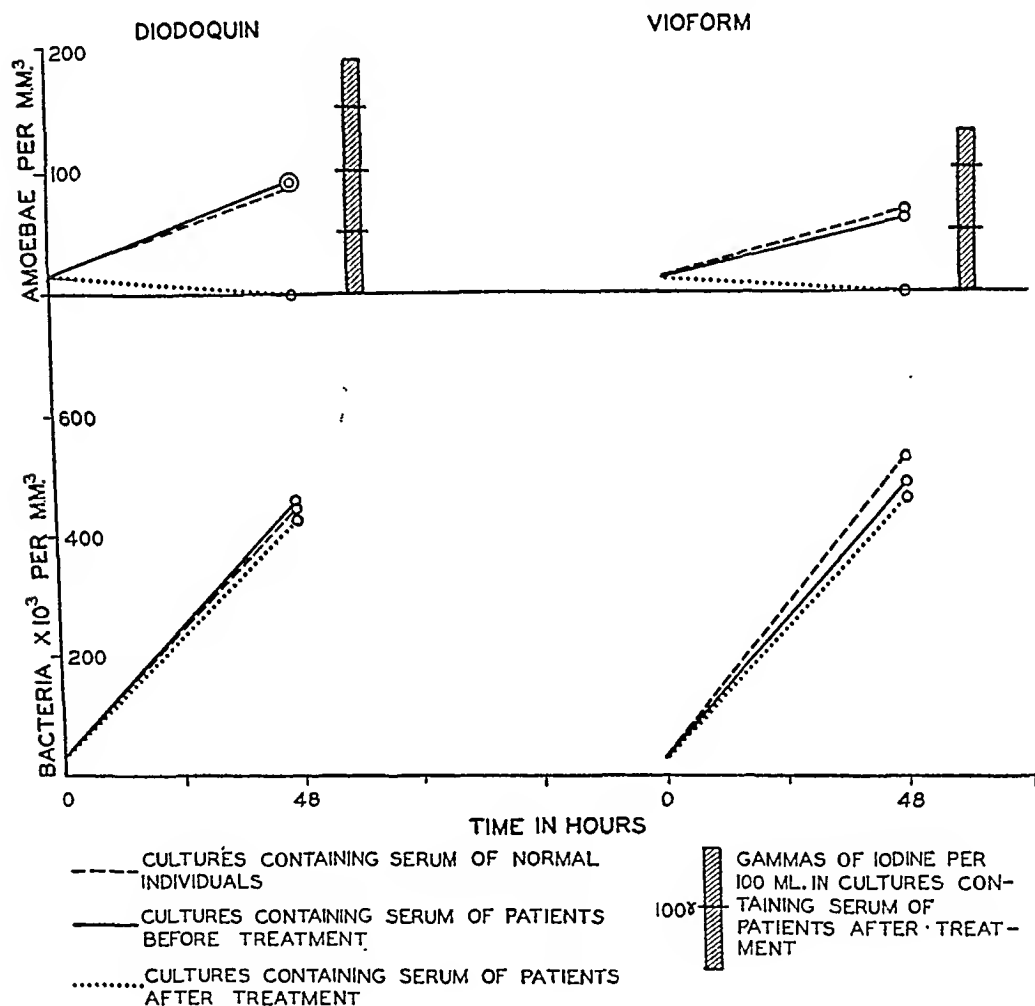


FIG. 1

plated out on nutrient agar. The plates were incubated at 37°C. for 24 hours, and counts were made with the aid of a plate counting chamber.

Iodine determinations on the sera after treatment were made by the method of Matthews, Curtis and Brode (4) with modifications previously mentioned in our earlier work (1).

Preparation of the stool specimens prior to iodine determination was as follows. The entire stool specimen was weighed and placed in a 2 liter beaker. The stool was thoroughly mixed with 25 to 50 ml. of water, and concentrated sulfuric

acid was then added slowly and carefully. A slow addition of sulfuric acid is important to prevent over-heating and charring and to insure uniformity of sample. The first 100 ml. of acid were added in 5 ml. amounts. Thorough mixing and cooling are necessary after each addition. The remaining sulfuric acid was added

TABLE II

Vioform

PATIENT	IODINE* IN CULTURE AFTER TREATMENT	AMOEBAE† PER MM. ³ AT 0 HOURS	AMOEBAE PER MM. ³ AT 48 HOURS			BACTERIA† × 10 ³ PER MM. ³ AT 0 HOURS	BACTERIA × 10 ³ PER MM. ³ AT 48 HOURS		
			Control	Before treatment	After treatment		Control	Before treatment	After treatment
H. S.	236	12	51	74	0	20	490	430	450
W. S.	300	10	75	42	0	23	590	480	450
A. B. J.	257	10	75	66	0	23	590	570	550
C. J.	299	13	68	88	0	15	410	430	470
J. S.	354	12	100	81	0	20	580	470	480
G. H.	140	10	89	65	3	25	620	520	630
W. H.	255	13	84	62	0	21	490	395	469
D. T.	239	11	82	66	0	16	316	317	285
J. R.	194	10	62	55	0	20	560	470	390
H. B.	251	10	52	0	0	24	640	750	550
E. U.	310	10	79	66	0	20	570	490	400
Averages.....	258	11	65	60	0	21	534	490	460

* In gammas per 100 ml. media in 1:1 serum-egg infusion with rice starch.

† Calculated from count of inoculum.

TABLE III

Chiniofon

PATIENT	IODINE* IN CULTURE AFTER TREATMENT	AMOEBAE† PER MM. ³ AT 0 HOURS	AMOEBAE PER MM. ³ AT 48 HOURS			BACTERIA† × 10 ³ PER MM. ³ AT 0 HOURS	BACTERIA × 10 ³ PER MM. ³ AT 48 HOURS		
			Control	Before treatment	After treatment		Control	Before treatment	After treatment
A. P. H.	100	8	51	47	24	15	560	490	590
R. S.	108	8	51	51	28	15	560	790	490

Anayodin

J. K.	106	8	51	63	16	15	560	530	570
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* In gammas per 100 ml. media in 1:1 serum-egg infusion with rice starch.

† Calculated from count of inoculum.

in larger amounts, increasing from 10 to 25 ml. until a total of 500 or 600 ml. were added. Final dilution with concentrated sulfuric acid to a volume of one liter was made the following day. Ten ml. aliquots of the stool-sulfuric acid mixture were used for the iodine determination and procedure from this point was identical with that for blood.

RESULTS

In 15 patients treated with Diodoquin (Table I, Fig. 1), the average 48 hour growth of *Endamoeba histolytica* in cultures containing serum before treatment was 90 amoebae per mm.³ After 7 days of treatment the 48 hour growth was 0

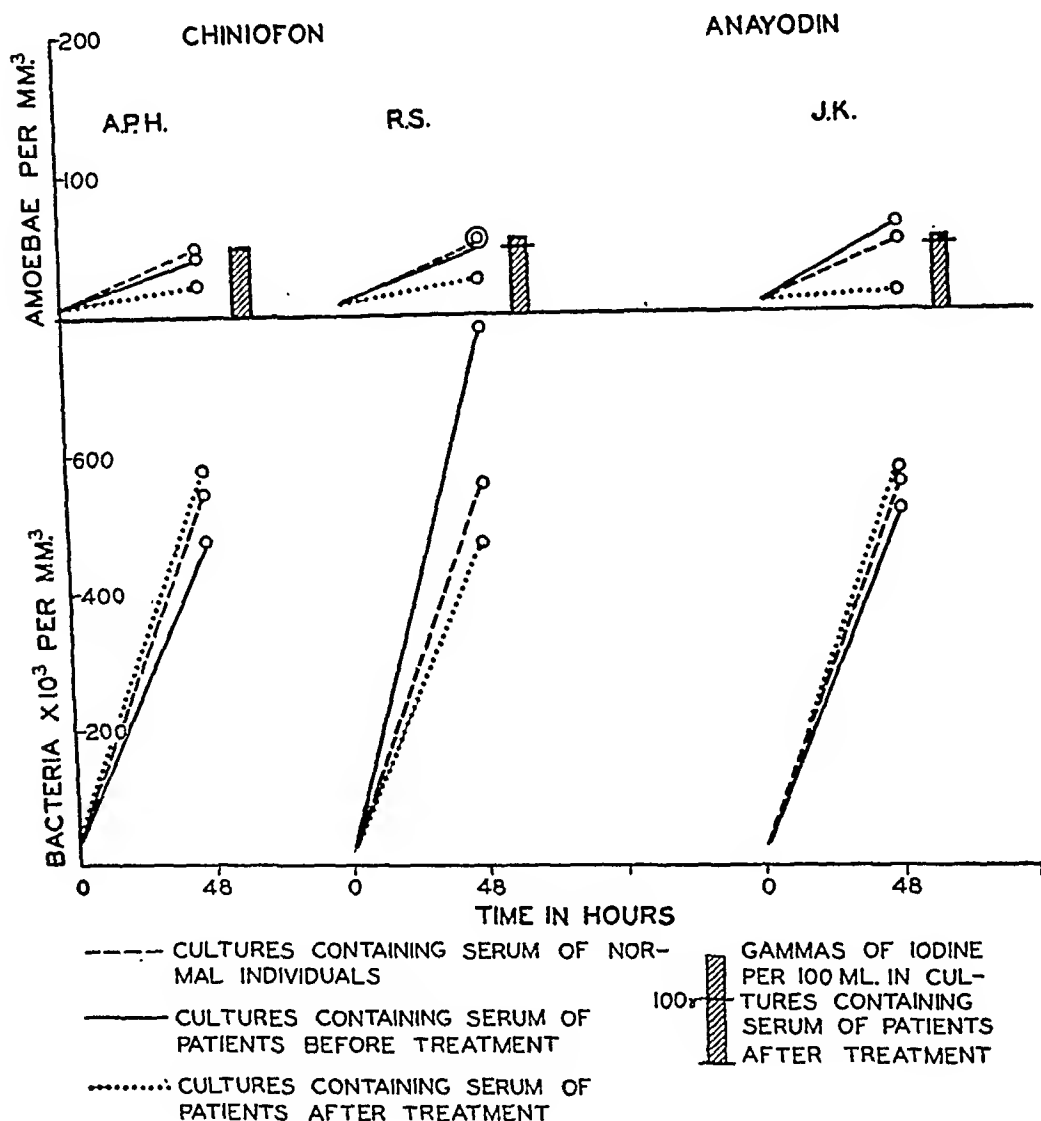


FIG. 2

in all except 3 patients, and these showed 1, 2, and 2 amoebae respectively. The media iodine levels were 185 gammas, 325 gammas, and 268 gammas per 100 ml. respectively. The average iodine level for all cultures was 380 gammas. One culture with 156 gammas and one with 214 gammas showed no growth. The control average growth was 89 amoebae. Bacterial growth was 445×10^3 per mm.³ before treatment and 430×10^3 after treatment. The control average was 440

TABLE IV
Diodoquin—serial dilutions

IODINE* IN CULTURE	AMOEBAE† PER MM. ³ AT 0 HOURS	AMOEBAE PER MM. ³ AT 0 HOURS	BACTERIA† × 10 ³ PER MM. ³ AT 0 HOURS	BACTERIA × 10 ³ PER MM. ³ AT 48 HOURS
Experiment 1				
0	14	91	14	402
84	14	20	14	397
169	14	11	14	389
253	14	0	14	329
339	14	0	14	346
Experiment 2				
0	18	77	16	430
84	18	26	16	500
169	18	11	16	358
253	18	0	16	420
339	18	0	16	361

* In gammas per 100 ml. media in 1:1 serum-egg infusion with rice starch.

† Calculated from count of inoculum.

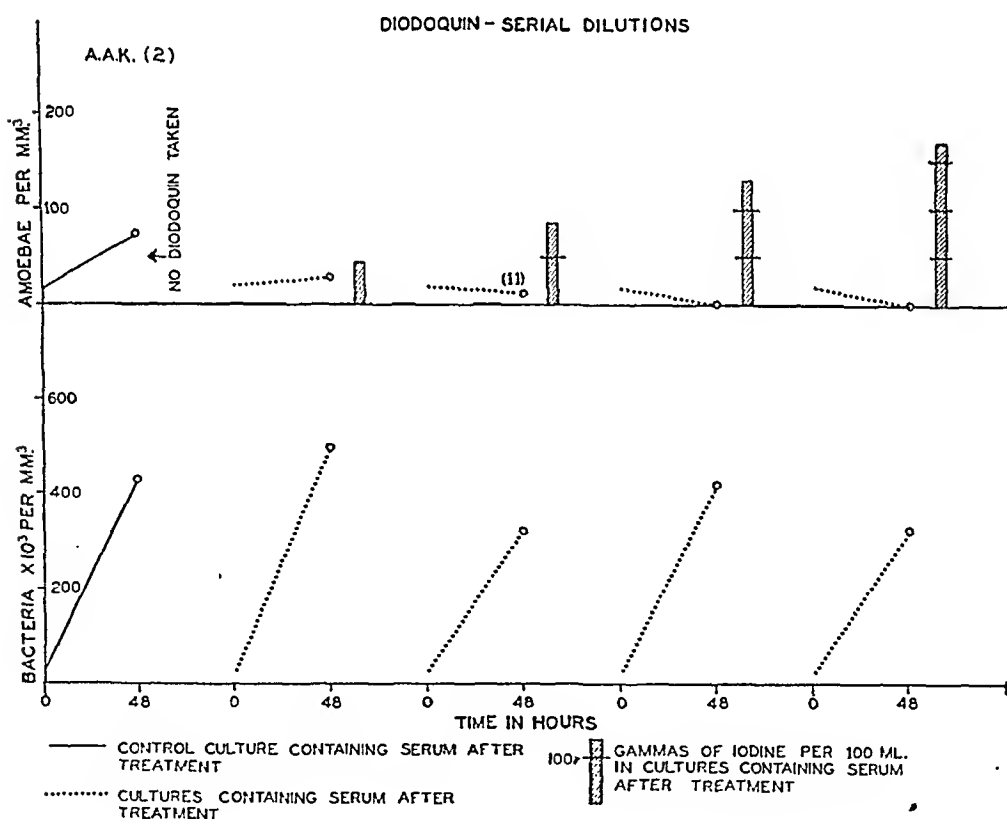


FIG. 3

$\times 10^3$. The average 0 hour count was 13 amoebae and 21×10^3 bacteria per mm.³

Vioform averages on 11 patients (Table II, Fig. 1) before treatment showed 60 amoebae per mm.³ at 48 hours. After 7 days of treatment the 48 hour growth was 0 in all except one patient, who showed 3 amoebae at 48 hours. The media iodine level was 140 gammas. The average iodine level for all cultures was 258 gammas. The average of the 11 controls was 65 amoebae per mm.³ The bacteria was 490×10^3 before treatment and 460×10^3 after treatment. The average of controls was 534×10^3 . The average 0 hour count was 11 amoebae and 21×10^3 bacteria per mm.³ One patient showed no amoebae growth in the culture containing serum before treatment.

TABLE V
Vioform—serial dilutions

IODINE* IN CULTURE	AMOEBAE† PER MM. ³ AT 0 HOURS	AMOEBAE PER MM. ³ AT 48 HOURS	BACTERIA† $\times 10^3$ PER MM. ³ AT 0 HOURS	BACTERIA $\times 10^3$ PER MM. ³ AT 48 HOURS
Experiment 1				
0	12	59	20	334
57	12	22	20	291
114	12	2	20	316
171	12	4	20	342
228	12	0	20	301
Experiment 2				
0	15	71	18	317
57	15	33	18	341
114	15	11	18	382
171	15	1	18	290
228	15	0	18	326

* In gammas per 100 ml. media in 1:1 serum-egg infusion with rice starch.

† Calculated from count of inoculum.

Two patients with Chiniofon (Table III, Fig. 2) showed 47 and 51 amoebae at 48 hours before treatment and 24 and 28 after treatment. The bacterial count was unchanged. The 0 hour count was 8 amoebae and 15×10^3 bacteria per mm.³ The media levels of iodine were 100 and 108 gammas in the cultures after treatment.

One Anayodin patient (Table III, Fig. 2) showed 63 amoebae at 48 hours before treatment and 16 after treatment. The bacterial growth was unchanged. The 0 hour count was 8 amoebae and 15×10^3 bacteria. The media iodine level was 106 gammas.

One subject who took Diodoquin (Table IV) volunteered 375 ml. of blood after taking the drug and from this and blood drawn before treatment, serial dilutions were set up in duplicate at levels of 0, 84, 169, 253, and 339 gammas of iodine. (Fig. 3)

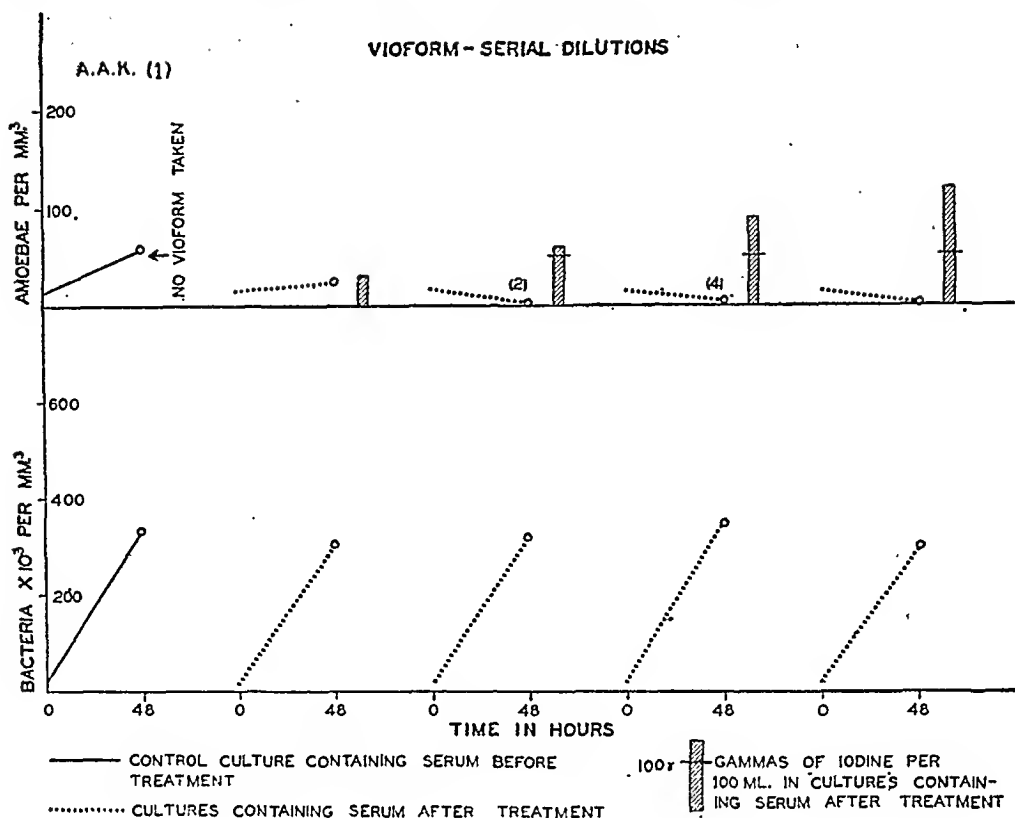


FIG. 4

TABLE VI
Diodoquin serum and stool iodines

PATIENT	SERUM* IODINE	WEIGHT OF STOOL <i>gms.</i>	STOOL IODINE†	
			Per entire stool	Per 100 gms.
B. S.	1252	201.5	358.5	173.0
M. D.	567	30.0	31.9	106.5
J. G.	844	15.8	95.0	602.9
J. M.	1163	233.8	617.9	264.4
J. V. A.	1220	64.0	96.8	151.3
J. A. T.	1474	275.0	353.0	320.9
H. T.	1721	165.0	431.4	261.5
A. O.	905	102.0	281.3	275.1
Averages.....	1143	135.9	283.2	269.5

* In gammas per 100 ml.

† In mgms.

One series with a 0 hour count of 14 amoebae and 14×10^3 bacteria showed growths of amoebae at 48 hours of 91, 20, 11, 0, and 0 and bacteria of 402×10^3 , 397×10^3 , 389×10^3 , 329×10^3 , and 346×10^3 while the other with a 0 hour

count of 18 amoebae and 16×10^3 bacteria showed at 48 hours, growths of 77, 26, 11, 0, and 0 amoebae and 430×10^3 , 500×10^3 , 358×10^3 , 420×10^3 , and 361×10^3 bacteria.

The same subject took Vioform (Table V) and serial dilutions were set up at levels of 0, 57, 114, 171, and 228 gammas of iodine. (Fig. 4) One series with a 0 hour count of 12 amoebae and 20×10^3 bacteria showed at 48 hours, growths of 59, 22, 2, 4, and 0 amoebae and 334×10^3 , 291×10^3 , 316×10^3 , 342×10^3 , and 301×10^3 bacteria. The other with a 0 hour count of 15 amoebae and 18×10^3 bacteria showed at 48 hours, growth of 71, 33, 11, 1, and 0 amoebae and 317×10^3 , 341×10^3 , 382×10^3 , 290×10^3 , and 326×10^3 bacteria.

TABLE VII
Vioform serum and stool iodines

PATIENT	SERUM* IODINE	WEIGHT OF STOOL	STOOL IODINE†	
			Per entire stool	Per 100 gms.
		<i>gms.</i>		
C. K.	433	71.0	95.8	129.4
J. G.	111	134.0	57.5	42.9
J. C.	341	36.0	17.4	48.4
R. L.	878	117.0	42.7	36.5
J. L. B.	182	202.0	98.1	49.0
G. F.	620	100.0	190.0	190.0
L. B.	505	71.0	24.2	34.1
F. M. R.	228	183.0	198.6	108.5
G. B. O.	224	114.0	170.8	149.8
M. L. B.	575	183.0	198.4	102.9
K. S.	466	85.5	53.3	62.3
P. S.	302	141.0	112.8	80.0
A. M.	677	153.0	70.5	46.1
M. H.	321	182.5	228.8	125.3
Averages	419	126.6	111.4	86.1

* In gammas per 100 ml.

† In mgms.

Eight patients after 7 days of Diodoquin (Table VI) showed serum levels averaging 1143 gammas per 100 ml. and stool levels averaging 269.5 mgms. per 100 gms. of stool.

Fourteen patients after 7 days of Vioform (Table VII) showed serum levels averaging 419 gammas per 100 ml. and stool levels averaging 86.1 mgms. per 100 gms. of stool.

SUMMARY

A new method of study in vitro of the therapeutic effectiveness of the oxyquinoline drugs, Anayodin, Chiniofon, Diodoquin, and Vioform on *Endamoeba histolytica* has been attempted.

This was done by inoculating human serum-egg infusion media with known

quantities of *Endamoeba histolytica* and accompanying bacteria and determining the amount of growth at 48 hours. The first media contained serum obtained from the patient before taking the drug, the second contained serum of known iodine concentration obtained after 7 days treatment with the drug, and the third contained serum from a non-amoebic, non-treated control.

The sera from 15 patients taking Diodoquin, 11 patients taking Vioform, 2 taking Chiniofon, and 1 taking Anayodin were studied.

Serial dilutions were made of both Diodoquin and Vioform containing sera and the amount of growth at 48 hours determined.

The oxyquinoline drugs in the human sera appear to inhibit the growth of *Endamoeba histolytica in vitro* in inverse proportion to the iodine concentration. The concentration necessary to cause disappearance from the media of all amoebae at 48 hours apparently lies between 250 and 300 gammas.

Seven patients on Diodoquin for 7 days showed stool iodine concentrations approximately 250 times the serum concentrations of iodine. Fourteen patients on Vioform for 7 days showed stool iodine concentrations approximately 175 times the serum concentrations of iodine. Three patients on Chiniofon and Anayodin showed stool concentrations of approximately 425 to 700 times the serum concentrations. Control of all tendency to diarrhea enhances the absorption in man of the oxyquinoline drugs, Anayodin, Chiniofon, Diodoquin, and Vioform.

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CHRONIC AMEBIC ABSCESS OF LIVER AND ASPIRATION LIVER BIOPSY

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Surgical treatment of chronic single amebic abscesses of the liver still carries with it a definite surgical risk and a certain mortality. In most text books the treatment of choice recommended for such abscesses is repeated aspiration together with specific emetine injections. This treatment may be disappointing since repeated puncture in debilitated patients may cause secondary infection of the abscess. In chronic cases secondary infection may even appear to be present at the initial puncture. Especially in secondarily infected cases drainage of an amebic abscess is contraindicated.

Even in endemic areas the diagnosis of a large single amebic abscess of the liver may be difficult. Occasionally the typical tenderness of the enlarged liver, pain to pressure in intercostal spaces, restricted respiratory movements and even a significant leucocytosis may be missing. It is well known that under these circumstances *Endamoeba histolytica* frequently cannot be found in purged stool samples or in material obtained by sygmoideoscope. A history of repeated episodes of diarrhoea is too common in inhabitants of endemic areas to be of any significance.

Most large single amebic abscesses of the liver are situated in the right liver lobe, extending upward and frequently involving the diaphragm, giving rise in such cases to irritation of the basal pleura, causing unproductive cough, respiratory pain and at times effusion in the phrenico-costal sinus.

The deeper the situation of the abscess the less evident are the above symptoms. Craig and Faust (1) mention the fact that the larger and the more chronic the abscess the lower the white blood cell count. In case the abscess does not extend upward fluoroscopic and radiological signs may also be absent.

In such cases only some tenderness in the right hypochondrium in the presence of hepatomegaly and irregular but not necessarily intermittent fever in a patient complaining further about fatigue, anorexia, and weightloss point to a pathological condition of the liver. In two such cases where a liver aspiration-biopsy was done an amebic abscess was found.

Where an amebic abscess is suspected and where local edema of the chest wall and local intercostal tenderness are not present, the usually recommended site of election for an exploratory puncture is in the anterior axillary line, well below the diaphragm. The site we prefer for a liver aspiration-biopsy is according to Iversen and Roholm (2), later strongly recommended by Haex (3), in or slightly dorsal of the posterior axillary line slightly below the diaphragm and never caudal from the 9th intercostal space, always in extreme expiratory phase with the patient sitting up with half flexed thighs and knees. The puncture is then made through the sinus pleurae and the diaphragm in the dome of the liver.

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With a careful anesthesia technic and puncture, shock is never seen and damage to vital tissues is evaded. One of the advantages of this method is that where an amebic abscess is contaminated by cocci or *Escherichia coli* a secondary peritonitis is less likely to occur than a pleura infection.

Through the easily manipulated 15 gauge liver biopsy needle most of the abscess content can be aspirated. In both of our cases an instillation of penicillin, 500,000 units in 20 cc. was made through the needle into the abscess cavity and the needle was withdrawn while the 2 cc. of a 5000 units per cc. penicillin solution were deposited in the puncture canal.

Noth and Hirshfeld (4) in 1943 reported a case of a large single amebic abscess treated by repeated punctures and repeated penicillin instillation through a ureter catheter in addition to specific antiamebic therapy. They remark already that repeated punctures and repeated penicillin instillations are in many cases probably unnecessary.

Hargreaves (5) strongly recommended additional use of penicillin (and sulfasuccidine) in cases of severe amebic infestation and Willmore (6) had similar favorable experiences. In a recent brief survey of the treatment of amebic dysentery however Van Steenis (7) was not convinced of the effect of additional antibiotic therapy.

Considering the common opinion that closed treatment of an amebic hepatic abscess is by far preferable to open drainage, which latter approach is apt to cause secondary infection, and that consequently as few punctures as possible should be done, a single puncture with penicillin instillation will probably be the treatment of choice.

Instillation of emetine locally into the abscess cavity as was successful in the case of Berkman and Bargaen (8), is apparently well tolerated, and may contribute to the effect that a single puncture will suffice. Kuenen (9) did not hesitate to deposit 60 mgr. emetine in 30 cc. saline in the abscess cavity.

Recently we saw two patients with a large single amebic abscess of the liver, which did not present the classic signs and was found at aspiration liver biopsy.

A 40 year old coloured Curacao pumpman, known to us for the last 13 years, complained in January 1948 about irregular, intermittent thoracic pain in relation to swallowing and somehow vaguely connected with gastric discomfort. There was no history of diarrhoea. At that time no cause could be found and we did not see the patient again until September 2nd 1948 when he had pain in both shoulders, fever, chilliness and tachycardia but no diarrhoea. There was a slight percussion dullness over the right lower pulmonary lobe but no cough. There was a non-tender hepatomegaly. The white blood cell count was 12,000 with only 1 per cent bandforms. The erythrocyte sedimentation rate was 100 mm. after one hour. The first 8 days of hospitalisation patient had an irregular intermittent fever for which no cause could be found. Repeated examinations of the stools failed to disclose the presence of protozoa, cysts or leucocytes. Radiological examination of the thorax and upper abdomen revealed a normal diaphragm and a completely normal upper- and lower border of the liver. After the 9th hospital day the temperature remained normal and patient was discharged October 8th

1948 without complaints and anxious to resume his work. He still had a high erythrocyte sedimentation rate and a white blood cell count of 9,000.

He was re-hospitalised November 4th 1948 with a relapse of the febrile disease after resuming his work. He then complained for the first time about pain in the right hypochondrium. The liver was enlarged and tender to edge palpation, percussion and pressure in the intercostal spaces. The usual liver function tests gave normal results. Gastro-duodenal soundage revealed a normally functioning gall bladder. A cholecystogram however was negative. A thorax radiogram on Nov. 17th did not reveal any anomalies in lungfields or in position or shape of the Right hemi-diaphragm. The Right phrenico-costal sinus was clear (Fig. 1.)

With bedrest the elevated temperature receded gradually but an anemia developed. The serum bilirubin was 0.15 mgr. per cent. Alkaline phosphatase was 17 units. Cephalincholesterol flocculation test was negative. There was less than



FIG. 1. Case 1. Thorax radiogram on Nov. 17th. Clear lungfields and clear Right phrenico-costal sinus. No anomalies in position or shape of Right hemi-diaphragm.

5 mgr. bromsulfalein retention after 45 minutes (5 mgr/Kg dose). Cholesterol esters were 38.7 per cent of a total serum cholesterol content of 90 mgr. per cent. Prothrombin content of the blood was 64 per cent of normal.

After treatment with large doses of Vitamin K parenterally and a transfusion of fresh whole blood, a liver aspiration biopsy with a 15 gauge needle was performed in the right posterior axillary line just below the level of the well moving diaphragma. About 4 cm from the skin, greenish-grey, brown-tinged, partly sanguinolent pus was obtained. An amount of 237 cc. of this pus was drained. Through the same needle 1 grain emetine hydrochloride and 500,000 units of penicillin in 20 cc. were instilled in the cavity. An additional 2 cc. à 5,000 units penicillin solution was deposited in the puncture canal on withdrawing the needle. Patient was instructed to lie on the left side. He received a transfusion of 450 cc. citrated blood. Treatment was continued with daily 1 grain emetine injections subcutaneously for ten days plus ehiniofon and carbarsone by mouth in the usual dose, plus 3 hourly intramuseular penicillin injections of 60,000 units each.

Patient had a short reaction to the liver puncture, consisting of dyspnoea and pain in the right hypochondrium. The abdominal wall remained supple. There was no vomiting and no cough. The prepuncture local anesthesia had been com-

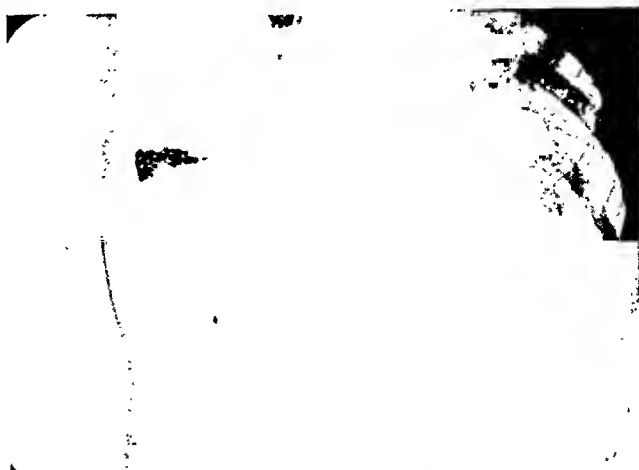


FIG. 2. CASE 1. Thorax radiogram one week after puncture. Air-bubble with fluid level below Right hemi-diaphragm. Density—probably extrapleural—on latero-posterior thoracic wall.



FIG. 3. CASE 1. 20 days after puncture. Thorax radiogram shows improvement in both thoracic and hepatic processes.

pletely adequate. The temperature which was between 38 and 39° on previous days rose .5 to 1 degree Celcius the next two days.

No amebae were seen in the direct eosin stained smear of the pus. Several gram positive cocci were seen in the Gram stained smear. On culture, hemolytic streptococci were recovered, which unfortunately, were not further determined.

From November 20th 1948 sulfadiazine was administered in addition for 7 days. From November 26th the temperature remained normal.

Chestplates in A.P. (Fig. 2) and right posterior oblique position taken Nov-

ember 26th, one week after the puncture, showed a triangular air bubble with a straight horizontal base and its apex .5 cm below the level of the diaphragm, situated within the denseness of the liver. Secondly there was a semi-circular area of density with its base on the lateroposterior thoracic wall, probably extra-pleural. The heart and mediastinum were not displaced.

The further clinical course was uneventful. Penicillin treatment was discontinued December 12th 1948. After 22 days radiological control on December 9th (Fig. 3) showed improvement in both thoracic and hepatic processes. On January 4th 1949 only a thickened pleura could be seen on the chestplate (Fig. 4).



FIG. 4. CASE 1. Thorax radiogram 46 days after puncture reveals thickened pleura as only rest of thoracic and hepatic processes.

The erythrocyte sedimentation rate and white count decreased gradually toward normal values. Patient gained weight and could be discharged February 5th 1949 in excellent condition and without any complaints.

Our other patient was a 30 year old machinist from Bonaire, known to us for two years previous to hospitalisation. He had never had any serious disease or important complaints and had no history of diarrhoea previous to his acute illness in June 1948. June 16th he became acutely ill with chilliness, fever, sore throat, generalised pains, nausea, vomiting and watery diarrhoea. At that time we had an influenza B epidemic, presenting similar symptoms. When this patient 5 days afterwards still had fever, headache and backache but no more diarrhoea, he was hospitalised. He complained about midthoracic pain and nonproductive cough. The pulse rate was relatively slow. Liver and spleen were both palpable

but not tender. There was a slight anemia of 11 gm. per cent with 3.4 million erythrocytes; a leucocytosis of 24,200 and a shift to the left of 9 per cent band forms. The erythrocyte sedimentation rate was markedly elevated to 109 and 131 mm. after 1 and 2 hours (Westergren).

On June 26th a liver aspiration-biopsy was performed in the posterior axillary line just below the diaphragm. Typical amebic pus was recovered and 80 cc. were aspirated. Via the aspiration needle 500,000 units penicillin in 20 cc. were instilled together with 1 grain emetine hydrochloride. Intramuscular penicillin injections 60,000 units 3 hourly dose and subcutaneous emetine injections with chiniofon treatment by mouth were instituted.

No amebae were seen in the direct eosin stained smear of the aspirated pus. No micro-organisms were recovered on culture.

Following the aspiration of the hepatic abscess and the subsequent specific treatment the temperature receded promptly and patient recovered without any untoward reaction. July 12th he was discharged in very satisfactory condition, without any complaints and fit for duty.

These two cases confirm the great advantage of closed treatment of amebic abscesses of the liver and consequently of as few punctures as possible. The results of Ochsner and De Bakey (10) are very convincing in this respect. They had a fatality of 5.6 per cent in hepatic abscesses treated by closed method against 43.1 per cent in cases treated by open drainage.

Secondly, our cases confirm the advantage of approach through the dome of the liver, entering in the posterior axillary line through the sinus pleurae and the diaphragm.

Thirdly, additional penicillin therapy seems to contribute to an uneventful post puncture course. In this respect we agree with Kullman and Golden (11) who recommend penicillin treatment as an adjunct whenever secondary invading organisms are suspected, which is of course always the case in aspiration of a liver abscess.

SUMMARY

Occasionally single large chronic amebic abscesses of the liver do not present classic diagnostic symptoms. In two such cases a diagnostic liver aspiration-biopsy disclosed the abscesses, which were further treated by a single aspiration, instillation of emetine and penicillin in the abscess cavity and subsequent routine anti-amebic treatment with emetine, chiniofon and carbarsone with penicillin intramuscular injections as an adjunct. Both patients recovered completely. Puncture through the sinus pleurae and diaphragm in the posterior axillary line is recommended.

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THE EFFECTS OF ORAL ADMINISTRATION OF AUREOMYCIN, SULFATHIAZOLE, SULFAMERAZINE AND 4,4'-DIAMINO DIPHENYL SULFONE ON TOXOPLASMOSIS IN MICE¹

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In experimental studies, sulfonamide and sulfone derivatives have offered the best possibilities for investigation in the search for a specific chemotherapeutic agent for the treatment of toxoplasmosis. Sulfathiazole and sulfapyridine (1), (2) were the first of these compounds found to protect animals inoculated with fatal dosages of the R.H. strain of toxoplasma. It was then demonstrated (3) that the action of these two drugs as well as of sulfadiazine in this disease was suppressive, and that foci of virulent parasites remain in the brain of animals long after treatment has been stopped.

Sulfadiazine and several sulfone compounds were superior to sulfathiazole and sulfapyridine for the protection of experimentally infected animals (4), (5), (6), (7), (8), (9). Recently it was reported (10) that sulfamerazine, sulfathiazole, sulfadiazine and sulfapyridine prolong the survival time of the chick embryo infected with the R.H. strain of toxoplasma.

In contrast, several antibiotics have been found to be without value for the protection or treatment of animals experimentally infected with toxoplasma. Neither penicillin (11) nor streptomycin (12) protected rats and mice, respectively. Penicillin, streptomycin and aureomycin (10) failed to prolong the survival time of the infected chick embryo.

The studies reported here are a part of the work being conducted to determine the mode of action of certain antimetabolites in the suppression of growth of toxoplasma *in vivo*. The wide range of activity against microorganisms reported for aureomycin (13) suggested the trial of this antibiotic in toxoplasmosis of mice. The action of aureomycin is compared with that of sulfathiazole, sulfamerazine and 4,4'-diamino diphenyl sulfone.

METHODS

The R.H. strain of toxoplasma used in these experiments was obtained from the Army Medical Center, Washington, D. C. in 1946. The parasite is maintained in mice by serial passage at 5 to 6 day intervals. For this purpose mouse peritoneal fluid containing the parasite is diluted one to ten in 0.85 per cent saline and 0.2 ml. of this mixture is inoculated intraperitoneally into mice.

In the experimental studies peritoneal fluid containing the parasite was obtained from four to five stock mice and pooled. Dilutions of 1×10^{-1} , 1×10^{-2} , 1×10^{-3} , and 1×10^{-4} were prepared in 0.85 per cent saline. Six mice weighing 15 to 20 grams were inoculated intraperitoneally with 0.1 ml. of the first dilu-

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tion and this procedure was repeated for each of the succeeding dilutions using the same number of mice in each case. The L.D. 50 titer calculations were made according to the method of Reed and Muench (14).

During the experimental period mice were fed a standard diet which consisted of sucrose 3650 gm., casein 900 gm. (vitamin free), salt mixture 150 gm., Alphacel³ 50 gm., cod liver oil 75 drops, potassium iodine 75.6 mgm., niacin 70 mgm., pyridoxine hydrochloride 25 mgm., thiamin hydrochloride 25 mgm., riboflavin 35 mgm., calcium pantothenate 75 mgm., and choline chloride 5 gm. Test compounds were administered to the animals in this diet. Approximately 4 gm. of diet was provided for each mouse per day. Water was available at all times. Toxicity controls were maintained in each experiment.

EXPERIMENTAL RESULTS

Experiment 1. Experimental diets were fed to groups of mice for two days prior to inoculation. One group received the standard diet containing 0.1 per

TABLE I

*The effects of administration of aureomycin hydrochloride and of sodium sulfathiazole on the course of experimental toxoplasmosis in mice**

COMPOUND AND CONCENTRATION IN DIET (%)	PROPORTION OF MICE DYING AT DILUTIONS OF				L.D. 50 LOG TITER ON THE 9TH DAY
	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}	
Aureomycin 0.1%	1/6	1/6	0/6	0/6	<1.0
Sodium Sulfathiazole 0.1%	0/6	1/6	0/6	0/6	<1.0
Control	6/6	6/6	6/6	3/6	4.00

* Date of inoculation 1/26/49.

cent aureomycin hydrochloride⁴, the second group, the standard diet containing 0.1 per cent sodium sulfathiazole and the third group (control), only the standard diet. Feeding was continued through the ninth day following inoculation. At that time 87 per cent of the control animals were dead. In contrast only 8 per cent of the aureomycin- and 4 per cent of the sodium sulfathiazole-treated animals were dead (see Table I). The three remaining control animals died on the tenth day after inoculation. There was no evidence of toxicity due to the test materials.

The experimental diets were replaced by commercial animal food on the tenth day and the survivors were observed for continuation of the protective effects of the test compounds. During this post-treatment period deaths occurred with equal regularity in both groups of survivors. Those which had received the lowest dilution of the original toxoplasma-inoculum tended to die first, while those which had received the next highest dilution diet a few days later etc., until all were dead at the end of the twenty-fourth day following inoculation.

Experiment 2. In the second experiment one group of mice received a diet containing 0.2 per cent of aureomycin, the second group, a diet containing 0.1 per

³ Obtained from Nutritional Biochemicals Corp., Cleveland, Ohio.

⁴ Lederle Laboratories Division, American Cyanamid Co., New York, N. Y.

cent sulfamerazine and the third group (control), only the standard diet. Feeding was started on the day of inoculation and was continued through the ninth day, at which time 83 per cent of the control animals were dead. On the other hand only 8 per cent of the aureomycin-treated animals were dead and 21 per cent of those which had received sulfamerazine were dead (see Table II). The four remaining controls died on the tenth day after inoculation. Sulfamerazine did not appear to be toxic but the aureomycin toxicity controls showed ruffling of coats and loss of appetite.

TABLE II

*The effects of administration of aureomycin hydrochloride and of sulfamerazine on the course of experimental toxoplasmosis in mice**

COMPOUND AND CONCENTRATION IN DIET (%)	PROPORTION OF MICE DYING AT DILUTIONS OF				L.D. 50 LOG TITER ON THE 9TH DAY
	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}	
Aureomycin 0.2%.....	2/6	0/6	0/6	0/6	<1.0
Sulfamerazine 0.1%.....	2/6	0/6	3/6	0/6	1.15
Control.....	6/6	6/6	6/6	2/6	3.74

* Date of inoculation 2/10/49.

TABLE III

*The effects of administration of aureomycin, para-aminobenzoic acid and 4,4'-diamino diphenyl sulfone on the course of experimental toxoplasmosis in mice**

COMPOUND AND CONCENTRATION IN DIET (%)	PROPORTION OF MICE DYING AT DILUTIONS OF				L.D. 50 LOG TITER ON THE 10TH DAY
	1×10^{-1}	1×10^{-2}	1×10^{-3}	1×10^{-4}	
Aureomycin 0.05%.....	5/6	2/6	0/6	0/6	1.65
Aureomycin 0.05% plus para-aminobenzoic acid 0.05%.....	5/6	1/6	0/6	0/6	1.49
Para-aminobenzoic acid 0.05%.....	6/6	6/6	6/6	5/6	4.00
4,4'-diamino diphenyl sulfone 0.05%.....	0/6	0/6	0/6	0/6	0.00
4,4'-diamino diphenyl sulfone 0.05% plus para-aminobenzoic acid 0.05%..	6/6	5/5	4/4	1/6	3.23
Control.....	6/6	5/6	5/6	3/6	3.70

* Date of inoculation 3/6/49.

On the tenth day following inoculation the survivors were placed on commercial animal food. Deaths occurred at approximately the same rate in these two groups of mice. The last survivors died on the twenty-sixth day following inoculation.

Experiment 3. This experiment was performed, in part, to determine to what degree a lower concentration of aureomycin than those used in the first two experiments would protect toxoplasma-infected mice and also whether para-aminobenzoic acid would nullify the protective effects of this antibiotic and of 4,4'-diamino diphenyl sulfone.⁵ Six groups of mice were each fed one of the following experimental diets for a period of two days prior to inoculation: (a) standard

⁵ Supplied by Merck and Co., Inc., Rahway, New Jersey.

diet (control group), standard diet containing (b) aureomycin, (c) para-aminobenzoic acid, (d) 4,4'-diamino diphenyl sulfone, (e) aureomycin and para-aminobenzoic acid and (f) 4,4'-diamino diphenyl sulfone and para-aminobenzoic acid. Each of the compounds was present in the diet at 0.05 per cent concentration. Feeding was continued until the tenth day following inoculation. At this time the percentage of death in each of the above groups was (a) 79 per cent, (b) 29 per cent, (c) 95 per cent, (d) 0.0 per cent, (e) 25 per cent and (f) 66 per cent.

Eleven days after inoculation the control animals and those which had received para-aminobenzoic acid had all died. At this time the survivors in the remaining groups were placed on commercial animal food. Those which had received aureomycin alone or in combination with para-aminobenzoic acid died at approximately the same rate. The majority died by the 24th day following inoculation. One animal, fed the combination diet, survived until the 35th day.

Survivors which had received the combination of 4,4'-diamino diphenyl sulfone and para-aminobenzoic acid were all dead at the end of the twelfth day following inoculation. In contrast, there were no deaths in the group fed 4,4'-diamino diphenyl sulfone alone until the 21st day after inoculation. Eighteen of these mice died between the 22nd and the 26th days. The remaining six were alive and normal two months after inoculation. These represented the group which received the 1×10^{-4} dilution of inoculum.

DISCUSSION

The results presented here give comparative data on the effectiveness of antibiotic, two sulfonamides and a sulfone in the protection of mice inoculated with the protozoan parasite *Toxoplasma*.

Aureomycin administered in the diet was equally protective at 0.1 and 0.2 per cent concentration. When administered at 0.05 per cent its protective value was less. There was obviously no instance of eradication of the infection since all of the mice which survived the initial period of treatment died after the experimental diet was removed.

Aureomycin afforded protection equal to that of an equivalent amount of sodium sulfathiazole. On the other hand sulfamerazine was less effective than any of the compounds tested.

4,4'-Diamino diphenyl sulfone not only provided the greatest protection of any compound tested; its administration resulted in the survival of six of twenty-four animals for a period of at least sixty days. These six survivors represent those inoculated with the highest dilution of the organism. In other studies (unpublished) it has been found that this sulfone is effective in murine toxoplasmosis until the diet concentration reaches the range of 0.006 per cent.

In a previous paper (15) the antagonistic effect of para-amino benzoic acid for sulfathiazole was demonstrated *in vivo* using toxoplasma as an indicator of metabolic antagonism. This same antagonistic effect produced by para-aminobenzoic acid has been demonstrated here against a sulfone. The antagonism between para-aminobenzoic acid and 4,4'-diamino diphenyl sulfone has been reported for bacteria by others (16), (17).

SUMMARY

The protective properties of aureomycin hydrochloride, sulfamerazine, sodium sulfathiazole or 4,4'-diamino diphenyl sulfone have been demonstrated and compared by oral administration to mice inoculated with toxoplasma.

The sulfone provided a greater degree of protection than any of the others tested. Aureomycin and sulfathiazole afforded approximately equal protection while sulfamerazine was the least effective.

Since para-aminobenzoic acid failed to counteract the protective effect of aureomycin it appears that the mode of action of this antibiotic in preventing the death of toxoplasma-infected mice is different than that of sodium sulfathiazole or 4,4'-diamino diphenyl sulfone.

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TOXOPLASMOSIS IN PANAMA: REPORT OF TWO ADDITIONAL CASES¹

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Since 1939, when Wolf, Cowan, and Paige (1) first described toxoplasmosis as a distinct disease entity, increasing numbers of human infections have been recorded in the literature. The world's knowledge pertaining to this parasitic state remains sufficiently meager to warrant the addition of two proven cases. It is not our purpose to review the minutia of clinical and pathologic data so adequately covered in recent discussions (2, 3, 4). We do, however, wish to (a) reconfirm the concept that intra-uterine infection is a potent factor in congenital or infantile form of the disease, (b) draw attention to the fact that the Isthmus of Panama is a prominent endemic focus and (c) briefly discuss certain clinical and laboratory aids currently available for diagnostic studies.

REPORT OF CASES

Case 1. A 29 year old, white, male Staff Sergeant, USAAF, stationed at Howard Field, Canal Zone, accidentally fell into the waters of the Panama Canal from a small Army craft on 20 March 1947. His lifeless body was recovered approximately one and a half hours later and death was ascribed to drowning. He was born in Dallas, Pennsylvania and had been in excellent health. No further information is available.

Necropsy: Gross: Autopsy was performed 15 hours after death by Major J. G. Bennett, M.C., AUS. The body had been embalmed. It weighed 153 pounds and measured 70.5 inches in length. No abnormalities were discovered on external examination.

Small amounts of bloody exudate were found in the ethmoid sinuses and middle ears. The meninges were free of exudate. The brain weighed 1350 grams and the convolitional pattern was unaltered. No inflammatory, neerotic, granulomatous, or hemorrhagic lesions were discovered.

The neck organs were not remarkable. Firm fibrous adhesions obliterated the left pleural cavity. The firm, gray-red lungs presented a combined weight of 2640 grams. Large amounts of edema fluid escaped from the cut surfaces but there was no pneumonic consolidation, tuberculosis, or other lesions. Particles of oil and dirt were adherent to the mucosal surfaces of the trachea and bronchi.

The heart weighed 320 grams and showed no abnormalities of size or contour. No areas of hemorrhage, necrosis, or scarring were found within the red-brown myocardium and the ventricular walls were not hypertrophied. The endocardium and valve leaflets were free of lesions and the coronary arterial system was unobstructed.

The remainder of the examination disclosed no significant abnormalities.

Microscopic examination: Microscopic studies served only to confirm death due to drowning. In sections from the left ventricular myocardium, however, there was one large pseudocyst measuring 120 by 50 microns within a distended muscle fiber (Fig. 1). It contained myriads of ovoid, basophilic bodies not exceeding two microns in diameter. The bodies consisted of tiny masses of nuclear chromatin surrounded by thin rims of clear cytoplasm.

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No parabasal bodies or kinetoplasts were discovered. The pseudocyst was neither encapsulated nor segmented. It was located at significant distance from the nearest blood vessel and its presence was not associated with inflammatory reaction. No further parasites could be demonstrated in multiple sections from the same block or in additional blocks of heart muscle. A careful search of sections from the brain, liver, and spleen failed to reveal parasitization of reticulo-endothelial cells by toxoplasma or other agents. The eyes were not examined.

Comment: The cause of death was ascribed to drowning and the finding of toxoplasma can only be considered incidental and noncontributory. This case exemplifies asymptomatic or inapparent chronic toxoplasmosis first predicted by Weinman (5) in 1943 and first demonstrated by Tomlinson (6), in this laboratory in 1945. Since that time five similar instances

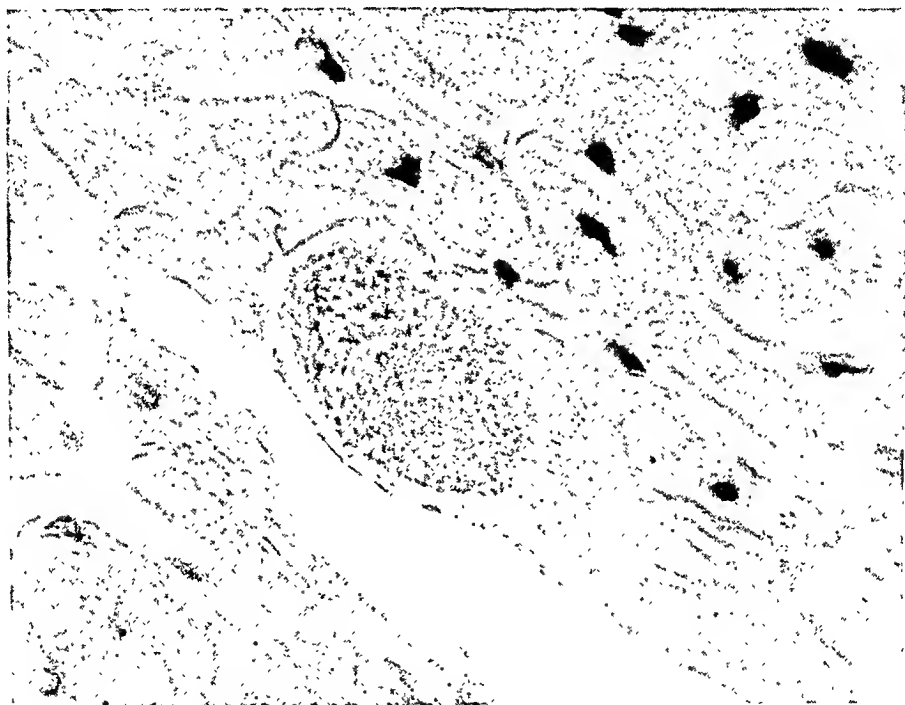


FIG. 1. CASE 1. TOXOPLASMIC PSEUDOCYST IN HEART MUSCLE

Note absence of inflammatory reaction. Oil immersion magnification.

have been added. The present represents the seventh case and the fifth to be discovered in Panama.

The complete absence of epidemiologic data prevents speculation as to the source of infection. No specific claim is made pertaining to its origin in Panama. The authors agree with Weinman (5) that inapparent chronic toxoplasmosis must be a relatively common affection. A study of this aspect is considered a fruitful field for future investigation in Central America.

Case 2. A 25 year old, gravida two, para zero, white female was first seen in the prenatal clinic of Margarita Hospital, Canal Zone, on 8 January 1948. Her last menstrual period was in September 1947. Physical examination, urinalysis, and complete blood count revealed no abnormalities and serologic tests for syphilis were negative. Her pregnancy had been uneventful thus far and her date of confinement was estimated as 6 May 1948.

The patient was born in England but spent much of her early childhood traveling

throughout Europe. Her past medical history was unremarkable except for mumps and acute appendicitis at the age of 15 years. In 1944, she suffered a severe attack of faucial diphtheria following which she had been predisposed to paroxysmal tachycardia and palpitation. In December 1946, the patient arrived in the Republic of Panama from England. She resided in the San Francisco district of Panama City with her husband. Her first pregnancy terminated in a spontaneous abortion in April 1947 during the ninth week of gestation. In December 1947, she moved from Panama City to the Margarita district of the Canal Zone.

Her husband was born in England but moved to Brooklyn, New York at the age of six. He suffered attacks of measles and pertussis in childhood. At the age of seven he was hospitalized with an ill-defined "kidney ailment." During puberty he suffered recurrent attacks of vague arthropathy and cutaneous abscesses. In 1943 he became a sailor and traveled extensively to Europe, the Mediterranean countries, Africa, and North and South America. He finally settled in the Republic of Panama in October 1946.

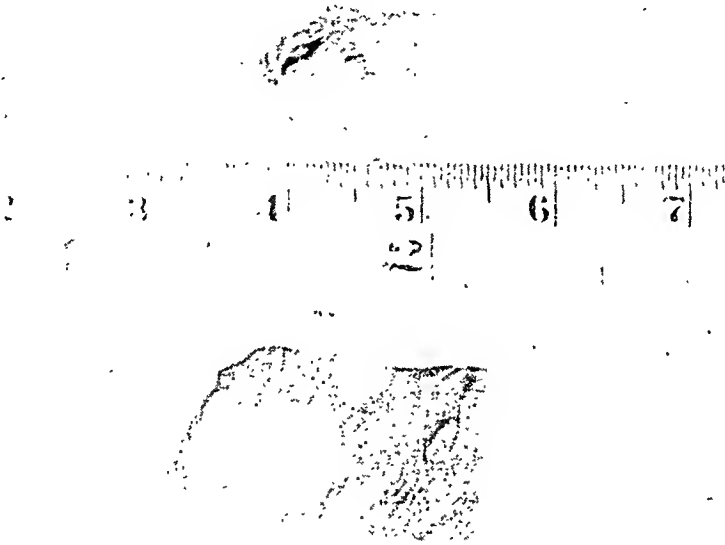


Fig. 2. CASE 2. PORTIONS OF BRAIN SHOWING YELLOW-TAN GRANULOMATOUS LESIONS IN CORTEX. APPROXIMATELY ACTUAL SIZE.

Conception of both pregnancies occurred while the parents resided in Panama City. Except for a dog belonging to a neighbor, animal contacts were denied. This beast was killed by an automobile shortly after the patient became pregnant for the second time. It had always enjoyed good health. Both parents specifically denied eruptive fevers or illnesses resembling atypical pneumonia.

During the afternoon of 15 April 1948, the patient experienced the spontaneous onset of recurrent abdominal pain. She was admitted to Margarita Hospital in active labor at 1:00 a.m. the following morning. Her temperature was 99.2 degrees F., but general physical examination was essentially negative. Although the patient had been conscious of fetal movements the previous day, none were palpable on admission and fetal heart tones could not be heard. Labor progressed without complication and she was delivered spontaneously of a stillborn female infant following a mediolateral episiotomy ten hours after admission. The placenta was expressed nine minutes later. The postpartum hospital course was uneventful.

The placenta was enlarged to approximately one-half the weight of the fetus. It was gray-brown and diffusely degenerated. The cotyledons were intact but enlarged, boggy, and edematous. There was diffuse yellow discoloration of the fetal surfaces. Amniotic fluid was copious and bright amber yellow. Abundant hemolyzed blood could be expressed from the umbilical cord. Unfortunately, the afterbirth was discarded without pathologic examination.

Necropsy: Gross: Performance of the autopsy was delayed 64 hours but the body was refrigerated between 32 and 38 degrees F. The deceased was a moderately macerated infant

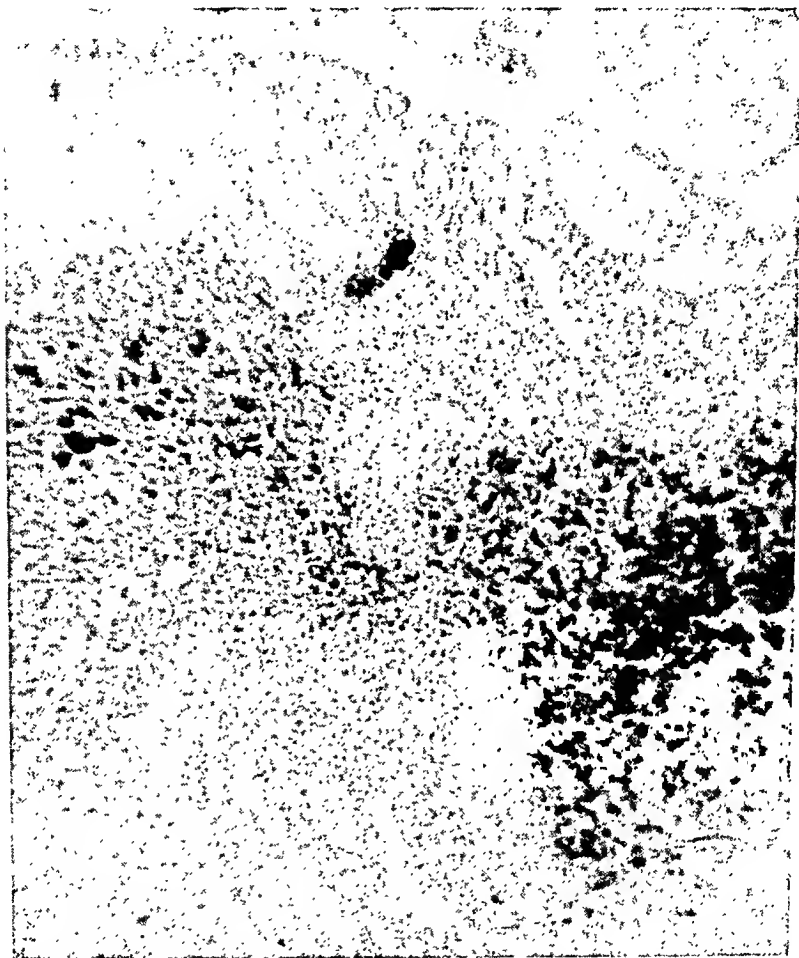


FIG. 3. CASE 2. SECTION FROM CEREBRAL CORTEX SHOWING MENINGITIS, CEREBRAL NECROSIS, AND CALCIFICATION. HIGH POWER MAGNIFICATION.

weighing 2700 grams and measuring 44.0 cm. in crown-heel length. There was intense pitting edema of all soft tissues. Mild scleral icterus, ocular proptosis, and abdominal distention were the only other notable findings on external examination.

The skull was well formed and of average size. The cerebrospinal fluid was grossly bloody but no source of hemorrhage was demonstrated in the cerebral envelopes. The leptomeninges were thickened, yellow-gray, and adherent to the cerebral cortex. Abundant gray exudate, chiefly in perivascular distribution, was contained within the subarachnoid space. Giemsa-stained smears of this material disclosed numerous intra- and extra-cellular lunate and rounded bodies strongly suggestive of toxoplasma.

The brain was remarkably softened and could not be weighed. The surfaces of the hemispheres were studded with irregular, yellow-tan, necrotic lesions, not exceeding 1.2 cm. in broadest dimension, penetrating the cortex to depths of 0.5 cm. (Fig. 2). The internal structure of the hemispheres was markedly distorted but no lesions were seen. The cerebellum, brain stem, and spinal cord were better preserved and contained no gross abnormalities. No choroid plexus tissue was recognized within the collapsed ventricles.

The eyes were excessively firm and the corneas were clouded. The lenses were opaque and softened. The aqueous chambers were slightly distended with cloudy gray fluid. The vitreous cavities contained gray-brown, gelatinous material. There was necrosis and swelling of the irides, retinal and choroid coats. The optic nerve heads were edematous.

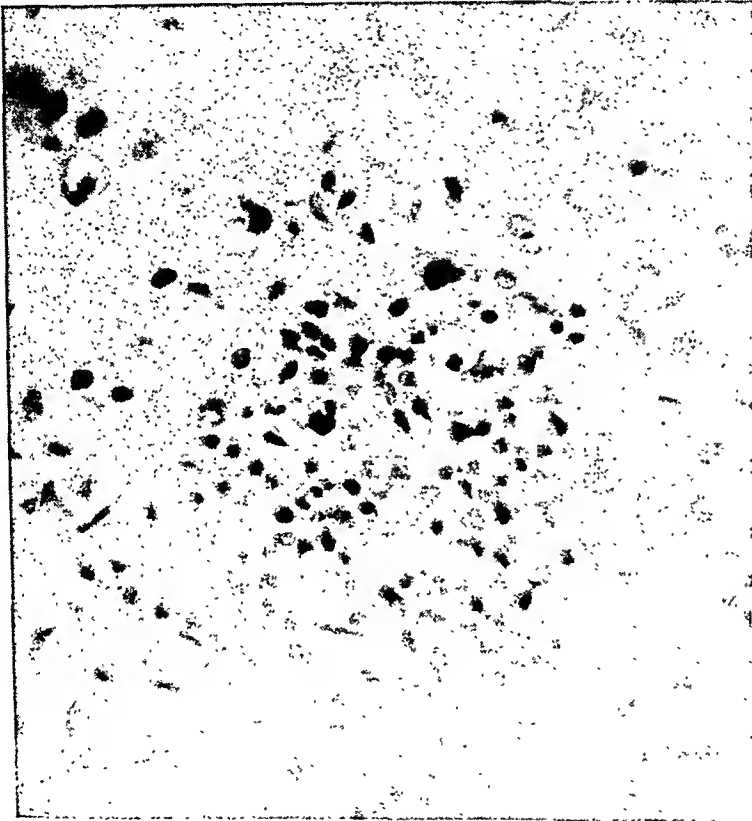


FIG. 4. CASE 2. GLIAL NODULE FROM MEDULLA. HIGH POWER MAGNIFICATION.

The pleural cavities each contained 20 cc. of hemolyzed bloody fluid. The lungs were nonaerated, edematous, congested, and together weighed 35 grams. The pericardial sac was distended by 10 cc. of hemolyzed bloody fluid. The heart weighed 13 grams and was soft, flabby, and dilated. No anomalies were discovered.

Edema of the peritoneum was prominent and 75 cc. of hemolyzed bloody fluid were present within the abdominal cavity. The liver weighed 110 grams. Its parenchyma was gray-brown, soft, and friable but contained no lesions. Noteworthy splenomegaly was observed. The organ was firm, deeply congested, and weighed 23 grams. Follicles could not be distinguished with the naked eye. The adrenals together weighed 2.0 grams. They were cystic and small amounts of fluid blood escaped from cystic spaces in the medullas. Except for postmortem autolysis, the remaining abdominal organs were not remarkable.

Microscopic Examination: Wide areas of necrosis infiltrated with lymphocytes and

macrophages were encountered in the cerebral cortex (Fig. 3). The overlying leptomeninges were edematous and similarly inflamed. The lesions contained rounded toxoplasmas, not exceeding 2 microns in diameter, singly, and in small clusters. Marked phagocytosis of these bodies had occurred. Irregular areas of granular calcification within the lesions were confirmed by von Kossa's stain. The rounded punctate appearance of the calcium particles suggested that degenerating toxoplasmas may have served as a nidus for their deposition. Tiny focal glial nodules containing lymphocytes and occasional isolated toxoplasmas were widely scattered throughout the basal ganglia, hemispheric white matter, brain stem, and spinal cord (Fig. 4). These were well circumscribed and did not exceed 350 microns in diameter. No lesions were found in the cerebellum, Beneath the floor of the fourth ventricle and at several levels throughout the spinal cord, were occasional isolated toxoplasmic pseudocysts unassociated with surrounding inflammatory reaction (Fig. 5). There was a scant



FIG. 5. CASE 2. TOXOPLASMIC PSEUDOCYST FORMED IN SWOLLEN GANGLION CELL BENEATH FLOOR OF FOURTH VENTRICLE.

Note absence of inflammatory reaction and nucleus in process of extrusion. Oil immersion magnification.

fibrinous exudate upon the ependymal membrane and one small glial nodule distended the ependyma lining the third ventricle.

A moderately intense chorioretinitis was discovered in both eyes and the optic nerves were inflamed and necrotic. Free toxoplasmas were identified with great difficulty and no pseudoecysts were found.

The atelectatic, hyperemic lungs exhibited a mild diffuse round cell infiltration without focal lesions or parasites.

There was mild, diffuse pericarditis and numerous areas of myocarditis. The latter were predominantly perivascular and manifest by necrosis with infiltration of lymphocytes and macrophages. Parasites could not be found within these lesions.

Marked postmortem autolysis of the liver and spleen had occurred but areas of hematopoiesis could be distinguished. The spleen was extremely hyperemic. Extensive calcification of the renal tubules was the only abnormality found within the kidneys. The remaining organs were extensively autolyzed.

Large toxoplasmic pseudocysts were found within striated muscle fibers from the tongue, shoulder girdle, retroperitoneal tissues and right knee (Fig. 6). These varied approximately from 50 to 150 microns in length and 25 to 50 microns in width. They contained up to 300 organisms but seldom distended the cells in which they were contained. No inflammatory reaction was incited by their presence.

Many sections from the umbilical cord contained no abnormalities. The umbilical vessels as well as the vessels of nearly all organs contained numerous nucleated erythrocytes.

Animal Inoculation: A piece of fresh brain tissue macerated with five volumes of isotonic saline was injected intraperitoneally into a guinea pig. When the animal was sacrificed 15 days later, the abdomen was distended with yellow mucoid fluid which became

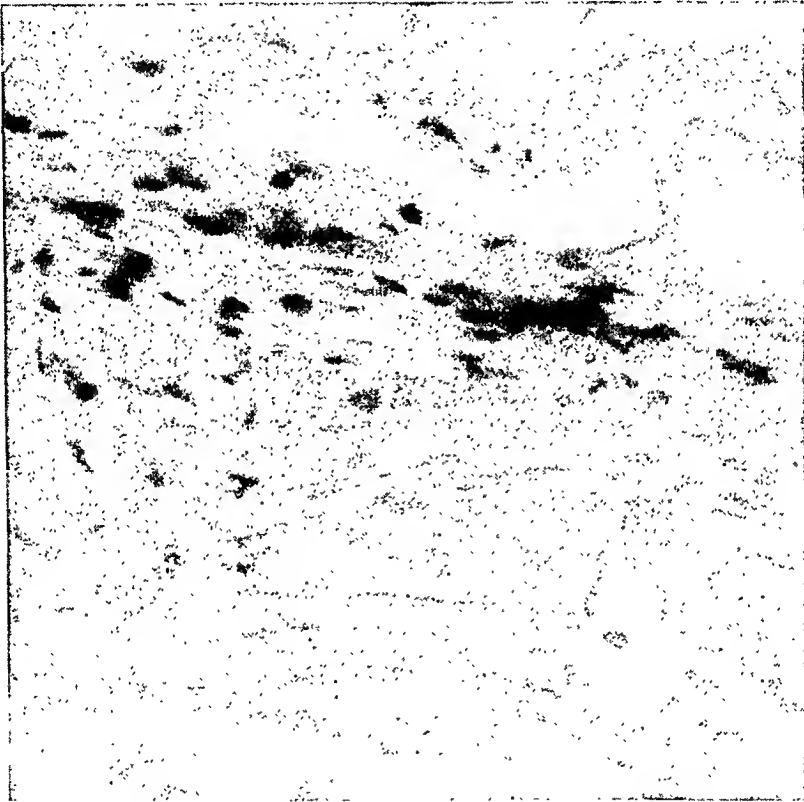


FIG. 6. CASE 2. TOXOPLASMA PSEUDOCYST IN FIBER FROM HAMSTRING MUSCLE. HIGH POWER MAGNIFICATION

more viscid on standing. The peritoneum was thickened, hyperemic, and velvety. Smears from the fluid and peritoneum contained typical crescentic organisms measuring 3×7 microns in broadest dimensions (Fig. 7). Their extremities were pointed and they contained rounded, eccentric, acidophilic, chromatin masses approximately two microns in diameter. Their cytoplasm was basophilic and faintly vacuolated. The associated peritoneal exudate consisted of lymphocytes and macrophages. Instances of phagocytosis were observed.

Microscopic examination of the organs disclosed a small granuloma containing free toxoplasmas within the brain. Pseudocysts were demonstrated in the heart, liver, striated muscle and renal tubular epithelium.

The strain was maintained in guinea pigs through three successive passages of peritoneal fluid. It is noteworthy that the animals did not succumb to the disease even when observed as long as 45 days. This recalls the experience of Rodaniche(17) who found the development

of clinical infection in inoculated guinea pigs required simultaneous inoculation with green producing streptococci.

Studies of the Parents: Both parents, as well as the child, were blood type O, Rh positive. Ophthalmoscopic examination of the parents failed to disclose chorioretinitis or other lesions. Skull films were not obtained.

Biopsies of striated muscle, obtained from both parents, were divided and one portion of each was macerated in isotonic saline and injected separately into the peritoneal sac of guinea pigs. The animal inoculated with muscle from the father survived 29 days; that from the mother, 50 days. Clinical disease was not produced in either animal and no evidence of toxoplasmosis could be demonstrated at autopsy. It is concluded that both cavies died of intercurrent disease.

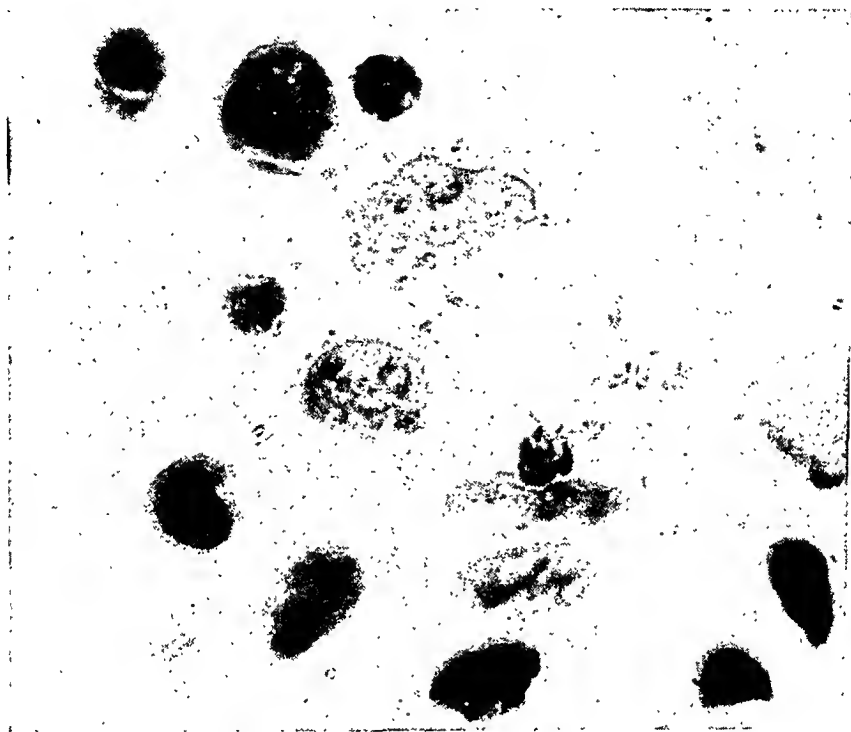


FIG. 7. CASE 2. FREE TOXOPLASMAS IN PERITONEAL EXUDATE OF INFECTED GUINEA PIG. Note crescentic shape and phagocytosis by a macrophage. Oil immersion magnification.

The remaining portions of biopsied muscle were examined by serial sections stained with hematoxylin and eosin. No "encysted" or free toxoplasma could be found.

Exactly four months after the birth of the child, skin tests were performed upon the parents using chick embryo toxoplasma antigen.⁵ The directions of Warren and Russ (7) were followed specifically, and 0.1 cc. intravermal skin test doses of 1/1000 and 1/100 dilution were utilized. Similarly prepared normal egg antigen was used as a control in intradermal dosage of 0.1 cc. of a 1/1000 dilution. The mother produced a positive erythematous indurated edematous reaction measuring 15.0 mm. in diameter to the 1/100 dilution in 24 hours. The father developed a similar reaction in 48 hours. Although a mild erythema measuring 4.0 mm. in diameter developed to the 1/1000 dilution in the mother, this could not be considered in the positive range.

⁵Obtained from Joel Warren, PhD, Army Medical Department, Research and Graduate School, Washington, D. C.

Complement fixation studies performed on sera from the father and mother using chick embryo vaccine, at the Army Medical Department Research and Graduate School were positive.

Comment: This case represents the second recorded instance of toxoplasmosis in a stillborn infant (8) and proves, beyond a shadow of reasonable doubt, that intra-uterine infection occurs. The lesions discovered in the central nervous system, heart, and skeletal muscle are considered typical and re-emphasize the innocuous nature of "encysted" toxoplasma confirmed consistently by all observers.

The association of soft tissue edema, scleral icterus, hemolyzed bloody effusions, erythroblastemia, and extramedullary hematopoiesis suggests a diagnosis of hydrops fetalis. Since no major or minor blood group incompatibilities were demonstrated, it appears unlikely that spontaneous iso-agglutinins could be a factor. Erythroblastosis fetalis with toxoplasmosis has been recorded by Steiner and Kaump (9). It is interesting to speculate that toxoplasma may elaborate a hemolytic toxin with particular affinity for the erythrocytes of the human fetus resulting in the same tissue responses noted in hemolytic disease of the newborn.

No reference to renal calcification is discovered in previously reported cases. This evidence of tubular damage may have resulted from the action of toxoplasma but it is equally likely that excretion of products of destroyed erythrocytes may have been the initiating factor. Similar calcifications are described by Yater and Mollari (10), and by Tomlinson (11) in patients dying of sickle cell anemia.

DISCUSSION

Toxoplasmas were originally described independently by Nicolle and Mancaux (12) as a naturally occurring infection in the African gundi and by Splendore (13) in a Brazilian rabbit. The parasite has been found with great frequency in lower animal forms, and infections in animals have been discovered in every hemisphere, continent, and climate.

A host of laboratory animals have proven suitable for transmission studies. Wolfson (14) and, more recently, Warren and Russ (7), have found chick embryos adaptable for culturing the parasites.

In Panama, Johnson (15) demonstrated toxoplasmosis among U. S. Army pigeons dying of an epidemic form of the disease. Asymptomatic toxoplasmosis occurred naturally in five of sixty laboratory guinea pigs examined by Kean and Grocott (16). These animals had been obtained from distributors in the United States. Feldman and Grocott, in an unpublished study, discovered toxoplasma in a dog dying of encephalitis. It was learned subsequently that the animal had been shipped to the Isthmus from Florida. Rodaniche and Pinzon (17) recently recorded the only naturally occurring animal infection (guinea pig) in Panama.

Other than the obvious intra-uterine infection of some human cases, nothing is known of the pathogenesis or epidemiology of the disease in man. Toxoplasmas have been found in the ovaries by Paige, Cowen, and Wolf (18) and in the testes by Zuelzer (19) in human cases. Lesions were demonstrated in the endometrium

of experimentally infected mice by Perrin (20). These observations suggest possible routes of human intra-uterine infections. The mode of transmission from maternal tissue to the fetus is not understood.

Sabin (21) states that the infection has been transmittent by feeding experiments and by intratracheal insufflation. Perrin (20) describes lesions in the muscularis and serosal coats of the gastrointestinal tract of experimentally infected mice. These discoveries suggest the possibility of human infection through the ingestion of food contaminated by animal excreta or the inhalation of dust particles similarly contaminated.

Parasites have been seen rarely in the blood of infected animals and animal transmission has been accomplished by inoculations of blood from infected individuals.

It is unusual that so little attention has been paid to the existence of insect vectors in a disease in which all studies seem to indicate an animal host. Pinkerton and Henderson (22) procured definite evidence of tick bites in two adult human cases. Chatton and Blanc (23) were unable to demonstrate organisms by direct examination or animal inoculations in two species of ticks, two species of mosquitoes, one species of mite, and one flea species collected from naturally infected gondi.

It seems likely that natural infections may occur through a multitude of channels with or without intermediate insect vectors.

In attempting to analyze the epidemiologic aspects of our second case, no definite conclusions can be drawn. It is noted with some interest that the parents were living in the San Francisco area of Panama at the time of conception. This district is within a five mile radius of Rio Abajo from whence came one of the patients discussed by Kean and Grocott (24). It is likewise observed that the guinea pigs found to be naturally infected with toxoplasma by Rodaniche (17) were obtained from the same general vicinity. Guinea pigs are frequently kept in homes as pets or for food by lower class Panamanians. Both parents were casually in contact with a dog owned by neighbors.

In a disease as little understood as toxoplasmosis, the absolute diagnosis depends upon the demonstration of the organism which is, apparently, an obligate tissue parasite. Toxoplasmas are typically crescentic but exhibit moderate pleomorphism. Average dimensions are 4 by 7 microns, and a fairly well defined mass of nuclear chromatin is usually located adjacent to the more pointed extremity. Reproduction is by binary fission and, as the parasite matures, it tends to become oval and round. Purposeful motility is denied by most observers.

Cross (25) postulates a life cycle of cellular invasion and multiplication until the capacity of the host cell to produce nourishment is exhausted. This is followed by destruction or extrusion of the host cell nucleus and ultimate rupture of the cell membrane freeing parasites for additional invasions. It is apparent that the membrane of parasitized cells may persist for indefinite periods during which the organisms remain in a resting state. The syncytial appearance thus produced is frequently misinterpreted as schizogeny.

Differentiation from *Sarcocystis*, *Encephalitozoon*, *Leishmania*, and *Histoplasma*

is required but is usually not difficult. These features are fully discussed by Perrin (20), Weinman (26), and Callahan *et al.* (2).

The parasites in tissue sections are altered by shrinkage and seldom exceed four microns in diameter. They frequently assume ovoid, rounded, and semi-lunate outline and often the nuclear chromatin is all that is visible. All tissues, with the possible exception of bone and cartilage, are susceptible to invasion but there is an apparent predilection for the nervous system, reticulo-endothelial system, and muscle.

The lesions produced vary considerably with the age of the subject. In infants and young children the nervous system and eyes are chiefly affected. The lungs, heart, skin, and possibly the eyes bear the brunt of the disease in older individuals. The inflammatory reaction is chronic, granulomatous, and often juxta-vascular. Necrosis of tissue occurs particularly in the central nervous system where it is almost invariably accompanied by calcification.

It is a noteworthy fact that only the free parasites stimulate an inflammatory response. Cells invaded by organisms to form pseudocysts are seldom surrounded by inflammatory exudate until disintegration occurs. Weinman (5) explains this feature with the hypothesis that decreased permeability of the host cell membrane prevents outward diffusion of metabolic wastes and toxins. A decrease in permeability has since been demonstrated by Cross (25) who discovered prolonged time requirement for hydrolysis in Faelgen's reaction in parasitized cells.

The geographic distribution of recorded infections proven by demonstration of the organism is outlined in the following table.

New York(1, 4, 18, 27).	8
Missouri(2, 22).	7
Panama(6, 16, 24, 28).	6
Brazil(29, 30).	3
Michigan(9, 19).	3
Ohio(9, 31).	2
Illinois(32).	2
South Carolina(3).	1
West Virginia(32).	1
Czechoslovakia(33).	1
Holland(34)	1
Peru(35)	1

The two cases presently described constitute the thirty-seventh and thirty-eighth in which organisms have been demonstrated and the seventh and eighth time they have been found in human disease in Panama. This represents an incidence of 21 per cent and is considered statistically significant. In reviewing the literature it is noted that the father of one patient described by Paige, Cowen, and Wolf (18) had been a soldier in Panama and the parents of a second case were born in the British West Indies.

From the clinical aspect, toxoplasmosis is invariably described as occurring in four main forms. We feel that the addition of a second case in a stillborn to that previously described by Paige, Cowen, and Wolf (18) justifies the segrega-

tion of a large group of infantile infections into a separate category. We therefore offer the following classification for the disease.

1. A congenital form with infection *in utero* manifest by fetal meningo-encephalomyelitis resulting in stillbirth or early neonatal death. Into this category fall the largest group of cases thus far discovered.

2. A congenital or acquired infantile type, frequently asymptomatic until later infancy or early childhood when the residua of previously inapparent lesions become manifest by visual disturbances, hydrocephalus, convulsive disorders or mental retardation. It is not clear at this time whether or not infection occurs *in utero*, during passage through the birth canal, or is acquired shortly after birth.

3. Acute encephalomyelitis in older children, presumably acquired, of which there are only two instances, both recorded by Sabin (31).

4. An acquired acute febrile form in adults characterized by a cutaneous eruption resembling the typhus-typhoid group of diseases with lesions demonstrable chiefly in lungs, heart, liver, spleen, and striated muscle. To date, six such cases have been discovered; three by Pinkerton and associates (22, 35), one by Darling reviewed by Kean and Grocott (28), one by Guimarães (29), and one by Syverton and Slavin (27).

5. An asymptomatic infection detected by immunologic methods, most often seen in the maternal parent of children with clinical disease, or discovered at autopsy as a purely incidental finding. Of this group, six instances have been recorded by Tomlinson (6), Gilmore *et al.* discussed by Kean and Grocott (28), Plaut (two cases) (36), and Kean and Grocott (two cases) (28).

The presence of neutralizing antibodies has been demonstrated (37, 38) but this immune factor is exceedingly heat labile. Other studies (39) have produced a complement fixation test using infected rabbit brain antigen. This method, however, is of questionable value in detecting chronic, healed or inapparent infection.

Recently Warren and Russ (7) have developed an antigen from infected chick embryos which may be used in both skin testing and complement fixation. Preliminary studies have indicated this agent to be both stable and reliable. Its successful use in the study of our case two, suggests its value in the diagnosis of inapparent infection.

SUMMARY AND CONCLUSIONS

1. Two cases of human toxoplasmosis are discussed. These increase the total number proven by morphologic demonstration of the parasite to thirty-nine.

2. One represents the second case of toxoplasmosis recorded in a still-born infant and reconfirms the concept that intra-uterine infection occurs.

3. A possible relationship between atypical erythroblastosis fetalis and congenital toxoplasmosis is postulated.

4. Attention is directed to the apparent high incidence of toxoplasmosis in Panama which seems to be a center of relatively high endemicity.

5. The current clinical classification of toxoplasmosis has been slightly modified.

6. Chick embryo antigen was used successfully to demonstrate inapparent toxoplasmosis in the parents of an infected stillborn infant.

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URINARY ENTERIC CARRIERS IN EGYPT: INCIDENCE IN 76 CASES AND OBSERVATIONS ON THE URINARY CARRIER STATE¹

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Studies on typhoid and paratyphoid fevers carried out by the United States Naval Medical Research Unit No. 3 in Cairo, Egypt, during 1948-49 afforded the opportunity to make some observations on the urinary carrier state in these diseases. In addition to acute cases studied on our ward, who developed the carrier state during convalescence, several other carriers were discovered. The validity of studies regarding the enteric disease carrier state are always subject to question unless scrupulous attention is paid to the bacteriological criteria and methods employed; further, temporary and chronic (duration over 12 months) carriers must be differentiated. This study is in the nature of a preliminary report since none of the cases were observed for longer than 13 months. Material presented in this report includes (a) study of carriers in 76 acute typhoid and paratyphoid patients, (b) bacteriological findings on 5 additional enteric carriers who were discovered and followed, (c) data on numbers of organisms excreted in the urine in several carriers and the effect of sodium bicarbonate on the bacilluria, and (d) results of treatment of 8 paratyphoid urinary carriers with sulfadiazine and streptomycin.

INCIDENCE OF CARRIER STATE DEVELOPING IN ACUTE TYPHOID AND PARATYPHOID FEVER PATIENTS

Selection of Cases: A total of 76 surviving typhoid and paratyphoid fever cases make up this series of patients admitted to our study ward during the acute stage of their disease. All patients were males, between the ages of 9 and 35 years, the great majority between 15 and 25 years, obtained from the third class (low-income group) general public wards of the Abbassia Fever Hospital in Cairo, Egypt. The cases were selected indiscriminately on the basis of positive blood cultures taken on new male admissions to the Fever Hospital who were regarded as possible cases of enteric fever. There was, of course, in a series of cases of this size considerable variation in the clinical courses. The majority of cases were first seen during the second week of disease, and exhibited a characteristic febrile pattern. The diagnosis by disease of the total group of 76 surviving pa-

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The opinions contained herein are the private ones of the writer and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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tients is given below:

Typhoid fever.....	45 cases
Paratyphoid A.....	28 cases
Paratyphoid B.....	1 case
Paratyphoid C.....	2 cases
Total.....	76 cases

Bacteriological Methods Employed: Organisms isolated by blood culture were identified on the basis of motility, biochemical reaction, and slide agglutination with specific "O" antisera for the typhoid and paratyphoid groups. Antisera used were obtained from the Standards Laboratory, Oxford, England.

Cultures of the feces by rectal swab and cultures of the urine were taken at intervals of one or more days after the patients had become afebrile before they were released. Three or more consecutive negative urine and stool cultures were used as the criterion for discharge of patients. Rectal swabs were streaked directly on Bacto SS agar (Difco) plates and then incubated for 18 to 24 hours in tetrathionate³ broth, a selective enrichment medium, for a second streaking on SS agar. The centrifuged sediment of urine cultures, collected in 25 by 150 mm. size potato tubes, was streaked directly on MacConkey's (Difco) agar and the sediment incubated for 18 to 24 hours in tetrathionate broth for a second plating on MacConkey's agar. Plates were inspected daily and suspicious colonies were picked and transferred to agar slants from which they were identified by motility, biochemical reactions, and slide agglutination with specific "O" antisera. In addition, many of the strains originally isolated from the blood were sent to the Enteric Pathogen Laboratory in charge of Commander L. A. Barnes, U. S. Naval Medical Research Institute, Bethesda, Maryland, for verification.

Incidence and Duration of Carrier State: It was found that of the total of 76 acute enteric fever patients, 9 continued to show the organism in the urine for varying periods of time during convalescence. The duration of the carrier state of these 9 patients and results of periodic cultures taken in following them is given in Chart 1.

In this paper the beginning of convalescence is defined as the first day the rectal temperature returned to and remained normal.

Five of the nine urinary carriers were para A patients, one of whom was still showing positive follow-up cultures in the 22nd week of convalescence (No. 59). The cultures of three para A patients reverted to negative after 6, 3 and 20 weeks respectively; one after sulfadiazine and streptomycin therapy (No. 55), another coincident with administration of sodium bicarbonate (No. 60), and a third, apparently spontaneously (No. 42). The final para A carrier could not be located for further observations after the 5th week of convalescence (No. 69).

The para C patient (No. 70) was still showing positive urine cultures in the 29th week of convalescence, at the time of this writing, as was the para B patient (No. 100), who was in his 17th week.

³ Barnes modification of Kaufmann's media, employed by Enteric Pathogen Laboratory, U. S. Naval Medical Research Institute, Bethesda, Maryland.

Week of Convalescence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	
Pt. 42 Para A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 55 Para A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 59 Para A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 60 Para A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 69 Para A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 70 Para C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 100 Para B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 111 Typhoid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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Pt. 112 Typhoid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Week of Convalescence	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	

CHART 1. CONVALESCENT DURATION OF URINARY CARRIER STATE IN 9 OF 76 ACUTE TYPHOID AND PARATYPHOID PATIENTS

The two typhoid cases (Nos. 111 and 112), who make up the remaining carriers cleared in the series of rectal patients, were observed only until the 9th and 10th weeks post-onset. At this time, one was found to have 4 successive positive urine cultures (subsequent stool cultures were negative) while the

TABLE 1

No.	CULTURE WITH MILLER-ABBOTT TUBE	RECTAL SWAB CULTURE
52	Attempted several times, but unsuccessful	6 negatives while urine cultures positive, and 11 more negatives when urines negative
53	Not done	7 negatives while urine cultures positive
54	Specimen after $MgSO_4$ administration and over-night specimen both negative while urine cultures positive	18 negatives while urine cultures positive
60	Specimen after $MgSO_4$ administration and over-night specimen both negative while urine cultures positive	12 negatives while urine cultures positive
69	Not done	6 negatives while urine cultures positive
70	Specimen after $MgSO_4$ administration and over-night specimen both negative while urine cultures positive	15 negatives while urine cultures positive
100	Attempted several times, but unsuccessful	16 negatives while urine cultures positive
111	Not done	7 negatives while urine cultures positive
112	Not done	3 negatives and 1 <i>positive</i> while urine cultures positive, and 9 negatives when urine culture negative.

other had apparently cleared on the basis of 9 successive negative urine and stool cultures.

The main emphasis in the bacteriological follow-up of the carriers was in the urine cultures. Nevertheless rectal-swab cultures were also taken in follow-up, but not so intensively. Investigation of the possibility that some of the patients may have been fecal as well as urinary carriers is summarized in Table 1.

Even though all of the nine convalescent urinary carriers may not represent

chronic permanent carriers, two points do stand out. First, although 45 of the original 76 cases were typhoid patients, only two developed the carrier state in convalescence, whereas the remaining seven carriers were from the 31 paratyphoid patients. Secondly, on the basis of the bacteriological methods employed, all of the carriers were urinary carriers. Only one (No. 112) had a single positive fecal culture, which occurred within the first 2 weeks of convalescence.

ADDITIONAL URINARY CARRIERS OBSERVED

Five additional paratyphoid urinary carriers, who were not observed during any acute stage of their disease, were discovered. The organisms involved were 3 para A's and 2 para C's. They were followed by urine cultures on three successive days each month, or more frequently, as well as by rectal-swab culture at irregular intervals. Bacteriological methods employed in the study of these cases were identical with the techniques used in following the acute convalescent carriers. Data pertinent to each of these 5 additional carriers is briefly summarized below.

Pt. No. 20. 20 year old Egyptian male. Acute febrile illness in March, 1948. Following this and thereafter for one year urine and stool cultures taken on three successive days each month consistently positive for paratyphoid C. Observed duration of carrier state at the time of this writing was 13 months.

Pt. No. 22. 30 year old Sudanese male cook. Discovered by routine urine culture. History of mild febrile illness several weeks previous. Repeated urine cultures consistently positive for paratyphoid A, and 4 rectal swab cultures negative during two weeks before treatment with combined streptomycin and sulfadiazine. Cultures became negative immediately following treatment, but follow-up urine cultures 5 months later positive again for para A on 3 successive days. Unable to locate patient for further follow-up.

Pt. No. 22A. 15 year old Egyptian male. Discovered by public health officials. Only 2 out of 3 urine cultures taken were positive for paratyphoid A before patient developed an acute febrile episode with severe abdominal and flank pain which was diagnosed as acute pyelonephritis. Treated with streptomycin and sulfadiazine after which follow-up urine cultures every month were negative for 8 months. One rectal swab culture before treatment and 9 more after treatment negative for para A.

Pt. No. 27. 28 year old Egyptian male cook. Discovered by routine urine culture. History of mild G-I symptoms without fever for previous 2 weeks. Urine cultures consistently positive for paratyphoid A before treatment with sulfadiazine and streptomycin. After treatment urine cultures negative. 14 weeks later urine culture again positive for para A and has remained positive for 8 months. 1 rectal swab culture before treatment was negative, and 16 more after treatment negative for para A while urine cultures positive.

Pt. No. 9465. 13 year old Egyptian male. Acute febrile illness one month before positive urine culture for paratyphoid C discovered. Urine cultures irregularly positive for two months, then treated with sulfonamides and urea during and after which urine cultures negative for one week. In last 4 months observed urine cultures consistently positive for para C at monthly intervals to the time of this writing. Total of 15 rectal swab cultures taken over 6 months' period of observation when urine cultures positive, two of these in last two months positive also for para C.

FURTHER OBSERVATIONS ON PARATYPHOID URINARY CARRIERS

Numbers of Organisms Excreted in the Urine: It is commonly stated¹ that urinary enteric carriers are likely to be a greater public health menace than fecal

excreters because of the large numbers of organisms which are passed in the urine. Garbat (2) has noted that urinary excretion may be intermittent; even to the degree that a specimen an hour before or after a positive culture may be negative. By using the technique of making pour plates with several dilutions of freshly voided urine in nutrient agar, it was possible to gain some idea of the numbers of organisms excreted in the urine of a urinary carrier. Urine cultures were taken before such determinations to rule out the possibility of concurrent bacilluria with other organisms, and questionable resulting colonies on the agar plates were checked by slide agglutination.

Values obtained from 4 paratyphoid carriers (2 para A's, 1 para B and 1 para C) on urine specimens 5 hours apart on successive days showed variations in numbers from day to day or in the same day. They were found to excrete anywhere from less than a hundred, or a few hundred to more than 50,000 organisms per ml. of urine. However, it was not unusual to find quite constant numbers on two daily specimens 5 hours apart over periods of 2 or 3 days. Values of over 10,000 organisms per ml. of urine were very common.

Effect of Sodium Bicarbonate: It was noted that daily urine cultures from a para A carrier (No. 55) became negative for three days while sodium bicarbonate was being administered to alkalize the urine previous to treatment with streptomycin. When bicarbonate was discontinued, the urine cultures promptly became positive again. The effect of giving 15 grams of sodium bicarbonate daily in divided doses was therefore tested in some of the other paratyphoid urinary carriers.

One para A carrier (No. 60) in the third week of convalescence reverted to negative when started on sodium bicarbonate, and has remained negative over 28 weeks of observation. Since he was only in the third week of convalescence it is likely that the association of bicarbonate and negative cultures was coincidental, and the patient would have become negative spontaneously.

In another para A carrier (No. 69) and a para C carrier (No. 70), however, sodium bicarbonate had no effect on positive urine cultures over a period of 5 days.

An attempt to quantitatively evaluate this phenomenon was made by measuring the pH of freshly voided urine specimens with a Beckman pH meter, and making pour plates for colony counts of numbers of organisms present in the urine as has been described above. Urine specimens were taken twice daily for five days before sodium bicarbonate was given, and for 5 days while on bicarbonate.

Under these conditions, one para A carrier (No. 27) showed no change in the number of organisms excreted while on sodium bicarbonate although the urine pH was altered from an average of 5.5 to about 7.75. Another para A carrier, however (No. 59), exhibited a marked decrease in organisms excreted, varying from 59 to >15,000 per ml. of urine before bicarbonate to <1 to 26 per ml. as the urine pH shifted from an average of 5.75 to 7.40. This was also observed in a para C carrier (No. 9465), who showed between 6 to >10,000 organisms per ml. of urine before bicarbonate was given, with a drop to <1 to 2 per ml. when

the urine pH rose from an average of about 5.6 to 7.7. The para B carrier (No. 100) showed a definite decrease in number of organisms in the urine also when receiving bicarbonate, though not so marked as the former two cases. The urine pH in this case changed from about 6.25 to 7.7.

The experience cited here of the effect of administration of sodium bicarbonate to enteric carriers is of further interest in view of the fact that Irwin and Houston (3) in 1909 reported the case of a chronic typhoid carrier receiving vaccine therapy in which urine cultures became negative after sodium lactate was given to make the urine alkaline. Also, Nichols (4), in 1917 advocated giving typhoid carriers sodium bicarbonate in the hope that further alkalization of the bile would not support the growth of typhoid organisms.

TREATMENT OF URINARY CARRIERS WITH SULFONAMIDES AND STREPTOMYCIN

The treatment of infections caused by the *Salmonella* group of organisms is generally considered to be ineffective with either sulfonamides or streptomycin. However, in view of recent favorable reports regarding the treatment of brucellosis with combined streptomycin and sulfadiazine (5, 6), this form of treatment was tried on some of the urinary paratyphoid carriers.

It was found that on full doses of sulfadiazine alone (4 grams stat and 1 gram every 4 hours), the urine cultures of two para A carriers became negative while on treatment, and for a few days after sulfadiazine was discontinued. This was also the experience with one para C carrier. However, urine cultures on these 3 patients again became positive.

Streptomycin alone for 7 days in doses of 0.5 gram every 4 hours with alkalization of the urine by sodium bicarbonate had no effect on the urine cultures in one para A carrier.

Combined therapy with the above doses of sulfadiazine and streptomycin every 4 hours for periods of 5 to 7 days was carried out on 5 para A and 2 para C carriers. In each of these cases, 12 to 15 grams of sodium bicarbonate in divided doses was also administered to maintain an alkaline urine.

Upon this regime the two para C carriers showed negative cultures for about a week, before they became positive again. One para A carrier (No. 22) had negative urine cultures for 3 days after combined therapy, but no further follow-up cultures could be obtained until 5 months later when they were found to be positive again. Another para A carrier (No. 59) had 2 separate courses of combined therapy which resulted in negative urine cultures for one week and one month respectively, before they reverted to positive. A third para A carrier (No. 27) showed negative urine cultures for at least 7 weeks after combined therapy, but was again found to be positive 15 weeks after treatment.

Possible cures resulted in two para A carriers. One of these (No. 55) had shown persistently positive urine cultures for 6 weeks after recovery from his disease. Following a 6-day course of combined sulfadiazine and streptomycin therapy follow-up cultures have remained negative for 28 weeks (see Chart 1). The other case (No. 22A) is less convincing in that he had been observed as a carrier for only 5 days before an acute episode of pyelonephritis with high fever

and flank pain demanded the institution of therapy. This patient has shown negative urine cultures for 8 months after treatment.

ASSOCIATED FINDING OF URINARY SCHISTOSOMIASIS

A simple positive correlation between urinary enteric disease carriers and the presence of urinary schistosomiasis in Egyptian native inhabitants of the lower Nile Valley would have no meaning in itself. The reasons for this are because the incidence of *Schistosoma hematobium* infestation is so high in this group of people and the number of urinary carriers which make up this report is too small to permit statistical evaluation. Yet it was striking to note that without special efforts or techniques being employed, ova of *S. hematobium* were demonstrated in 8 of the total of 14 urinary carriers on routine analyses of the urine while under observation. In addition, three of the remaining six cases gave a definite history of bilharzia previously. The other three cases had negative urines, and gave no past history indicative of schistosomiasis.

DISCUSSION

In an authoritative review of the problem of both fecal and urinary chronic carriers (1), it is stated by Browning that the majority of those patients who excrete organisms after six months will continue to do so. However, the chronic carrier is generally defined as one who still continues to excrete the organisms after a year following recovery from the disease (7, 8). Browning offers ample evidence for the existence of temporary carriers, especially following typhoid and paratyphoid B infections, who are carriers for varying periods of time up to one year after their illness. This has been the experience with paratyphoid B infections in England also (9, 10).

The incidence of chronic paratyphoid, as well as typhoid, carriers is believed to be between 2 and 5 per cent of all cases who have had the disease, although good statistics for the paratyphoid fevers are still comparatively scanty (1). Chief emphasis has always been placed on fecal carriers. Browning gives figures, for example, from a central enteric depot in England during World War I, which show only 1 urinary carrier and 15 fecal carriers out of 546 typhoid convalescents; 1 urinary and 23 fecal carriers from 837 cases of paratyphoid A; and 1 urinary and 23 fecal carriers after 1,425 cases of paratyphoid B. More recently, in an epidemic of paratyphoid A infection with 121 clinical cases, which occurred in Vienna in 1939, the organism was never found in the urine in over 2,500 specimens examined from cases or contacts (11).

However, there is evidence which indicates that the incidence of temporary urinary excretors is higher than is commonly appreciated. Bumke's (12) extensive experience with enteric carriers in the German Army during World War I showed a high incidence of bacilluria in convalescent typhoid and paratyphoid cases. Eleven of Garbat's (2) series of 164 male typhoid convalescents exhibited positive urine cultures one month after they were afebrile, but none persisted by the end of the third month. In the follow-up of a group of paratyphoid B

epidemic convalescents in England, Glass and Wright (13) have pointed out that positive urine cultures were much more frequent from the 6th to the 12th week after recovery than were positive stool cultures.

The series of cases reported in this paper is not large enough, and the duration of time during which the carriers were observed was too short to allow any definite conclusions to be reached regarding the true incidence of enteric disease carriers in Egypt. Yet, the experience presented in this report with 76 cases of typhoid and paratyphoid fever is sufficiently large to draw attention to the fact that of the 9 temporary carriers which resulted, every one was a urinary carrier. In only one, a typhoid patient, was the organism found in the stools. Undoubtedly some of this group of 9 convalescent urinary carriers belong to that class of temporary carriers who will clear spontaneously within 6 months or at most a year after their disease. But in at least 3 of the group the persistence of positive urine cultures for a period of 19 to 33 weeks is not conducive to optimism in this regard. If, for the sake of argument, the finding of negative fecal cultures were to be dismissed as being due to poor bacteriological technique, one is still confronted with the fact that all carriers showed organisms in their urine.

The 5 additional urinary carriers, who were discovered and studied but are not included in the group observed as acute cases, tend to support the apparent high incidence of urinary carriers in Egypt. The observed duration of the carrier states in all but one of this group varied from 5 to more than 12 months, so it is quite likely that all of these cases will become chronic carriers.

Another point is that all but two of the total of 14 urinary carriers reported here were caused by paratyphoid organisms. This is especially interesting in view of the fact that more than half of the acute cases observed were cases of typhoid fever. Thus in native Egyptian males of the low economic group, it would appear that paratyphoid organisms are more likely to involve the urinary tract than are typhoid organisms.

Because of the very high incidence of urinary schistosomiasis, or bilharzia, in Egypt it is interesting and worthwhile to speculate on the possible role which the manifestations of this disease might play in association with urinary enteric carriers. The pathology of the kidney in urinary enteric carriers may be a pyelonephritis, pyonephrosis or a perinephric abscess (14). Rupture of focal lesions into the kidney tubules is probably the main cause of the bacilluria (1). Pick (15) suggested that enteric organisms may multiply in the prostate or seminal vesicles in males, and in ducts of urethral glands in females. It has been pointed out that previous urinary tract disease or anomaly may often predispose to the urinary enteric carrier state (14). The possible significance of lymphatic communication between the ascending colon and right kidney in the etiology of typhoid urinary carriers is mentioned by Reimann (16). The pathologic changes produced directly by the ova in the lower urinary tract and the later secondary changes which result in the upper urinary tract could facilitate the establishment of enteric organisms in an already damaged urinary tract. Even though the evidence for active or previous infection due to *S. hematobium* was so readily

available in 11 of the 14 carriers of this report, the naturally occurring high incidence of schistosomiasis in native Egyptians of the Cairo area makes it difficult to evaluate the significance of this finding.

A study of 40 outbreaks of paratyphoid B infections in England has led Savage (17) to conclude that the role of the chronic carrier has been over emphasized in the epidemiology of the paratyphoid fevers. However, special features in the host environment of a vastly different geographical area and social group may demand important modifications of this concept. The implications of the data presented in this report, while admittedly preliminary, nevertheless suggest that further investigations along this line would be desirable and important from the public health standpoint.

SUMMARY

1. The incidence and follow-up data of the temporary urinary carrier state resulting in a series of 76 acute typhoid and paratyphoid fever cases in Egypt is presented.
2. Similar note is made on 5 additional non-acute paratyphoid urinary carriers who were discovered.
3. Observations on the numbers of organisms excreted in the urine and the effect of sodium bicarbonate on numbers of organisms excreted is given.
4. The relative ineffectiveness of combined therapy with streptomycin and sulfadiazine in several paratyphoid urinary carriers is noted.
5. The apparent high incidence of urinary enteric carriers in Egypt is emphasized and the possible etiologic association between urinary carriers and urinary schistosomiasis is suggested.

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Addendum: A recent communication from the United States Naval Medical Research Unit No. 3, in Egypt, reports an additional 11 weeks follow-up in Patients No. 20, 27, 70, 111, and 9465 to show urine and rectal swab cultures still positive as reported in this paper.

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LABORATORY DIAGNOSIS OF CHOLERA

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The clinical diagnosis of cholera, especially in the absence of an epidemic or in places where the disease is uncommon, is very likely to upset the public health of the local community, or even the nation, and can have serious repercussions in the trade and travel of the world. To avoid local and perhaps national apprehension, immediate laboratory confirmation of the clinical diagnosis of cholera is of utmost importance.

Selective media have been devised for the isolation of *Vibrio cholerae* which take advantage of the fact that it grows at a pH of 8.2 and higher whereas most other pathogenic and parasitic organisms are inhibited at this pH. The medium most commonly used has been Dieudonne's (1, 2) in which defibrinated beef blood and NaOH are added to agar. *V. comma* colonies grown on this medium have a slightly greenish tint. Aronson's medium (3) consists of nutrient agar to which are added sodium carbonate, saccharose, dextrin, alcoholic basic fuchsin and sodium sulfite. *V. comma* colonies are red in color, *E. coli* colonies are large but colorless. On Krumwiede's medium (4), which contains egg, sodium carbonate, peptone, agar and glucose, the *V. comma* colonies are hazy. Wilson, Blair and Read (5, 6, 7) used modifications of a medium containing bismuth sulfite in addition to beef extract, peptone, agar, saccharose, mannite, etc. The Vedder and van Dam medium (8) contains hemoglobin, peptone, glycol, potassium hydroxide and agar.

In the routine laboratory diagnosis of cholera during the 1947 epidemic in Egypt such complicated differential media for the isolation of *V. comma* proved to be cumbersome and time-consuming. We found that by using a simple nutrient agar to which enough alkali in the form of NaOH had been added to raise the pH to 8.4 we could increase our speed and efficiency in handling the numerous specimens that were being sent to the laboratory from patients and carriers. The following was the routine technique in isolating *V. cholerae*:

A test tube of peptone water, pH 8.4, is inoculated with several loopfuls of fresh stool and incubated for 8 hours. A loopful from the surface is examined by Gram stain, and for motility. Whether or not *V. cholerae* are found in the peptone, it is used to streak nutrient agar plates, pH 8.4, which are then incubated overnight at 37°. On the nutrient agar the colonies of *V. cholerae* are semi-transparent and look like droplets of dew while most of the other organisms are inhibited, and the ones that grow are opaque. The isolated organisms are streaked on agar slants and incubated for 12 hours, after which agglutination tests can be made. If speed is desired this step can be omitted and the *V. cholerae* from the alkaline nutrient agar emulsified in saline and agglutination tests carried out using anti-cholera O serum, dilutions being made serially and incubated at 37°C.

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overnight. If the organism is agglutinable it is tested for hemolysis. Tubes containing 2 cc. 5 per cent sheep or goat cells are inoculated with several loopfuls of a young culture, incubated at 37°C. for a few hours and inspected at intervals. If there is no hemolysis the organism is *Vibrio cholerae* (epidemic type, Inaba or Ogawa). If hemolysis occurs it will take place in stages of hemagglutination, hemodigestion and hemolysis, and characterizes El Tor vibrio.

Dr. Sven Gard, of the State Bacteriological Laboratory of Stockholm, Sweden, had developed a medium which he claims shortens the time necessary for diagnosis and is more accurate than routine procedures in detecting carriers. The method, which is still in the experimental stage, is introduced here for its possible interest to the bacteriologists in the United States. Twenty-five cc. of a semi-solid medium in a conical flask is used for the growth, isolation and agglutination of *V. cholerae*. The medium is prepared as follows:

Agar	0.35 Gm.
Peptone	3.00 Gm.
Water to	100.00 cc.,
heat to melt agar. Then add:	
5 cc. 0.1%	cadmium chloride
5 cc. 1.0%	potassium chlorate
1-2 cc. 1.0%	sodium hydroxide,
	and autoclave.

Twenty-five cc. of the medium is put in a sterile flask with a metal cover. A strip of filter paper about 2-3 mm. wide and 2½ cm. long dipped in 1:5 concentration of 3500 titer anti-cholera O serum is gently immersed on one side of the flask reaching almost to the bottom. On the other side of the flask about 1 Gm. of fecal material is introduced in a vertical manner by means of a pipette. The flask is incubated overnight, *V. cholerae* grow and, being motile, migrate from the column of the inoculum into the medium and become agglutinated around the filter paper, giving a cloudy appearance to that part of the medium. If there are no vibrio there is no cloudiness around the filter paper soaked in immune serum. According to the originator of this medium its chief advantage is the fact that a large inoculum (1 Gm.) is used instead of several loopfuls. There are thus more chances of recovering *V. cholerae* in the case of carriers using this technique than with routine methods of laboratory diagnosis. It is simple and saves time and labor.

In the 1947 cholera epidemic in Egypt we kept detailed records on 42 patients and 21 convalescent and contact carriers. Stool cultures were made regularly by our method. *V. cholerae* were found in the stools of patients on the 2nd (average) day of illness and were present in the stools for less than one day. Stools become and remained negative after the 5th (average) day. In the carriers also *V. cholerae* was detected in stools early and remained for less than 24 hours. Cultures were usually negative after 3½ days of observation.

For purposes of laboratory diagnosis *V. cholerae* can be isolated within the first 24 to 48 hours of illness, and occasionally as late as the 5th to 7th day. In the contact carriers also *V. cholerae* can be isolated soon after contact, but disappear

rapidly from the intestinal tract. One contact carrier whose stool cultures had been negative for 30 days had positive cultures on the 13th, 18th and 19th days of isolation. Such subacute or chronic healthy carriers in cholera are rare but dangerous, as they could easily be responsible for the spread of the disease from place to place, especially in these days of rapid transportation.

Of a total of 3,000 cases of contacts examined during the epidemic by laboratory methods, *V. cholerae* were found in the stools of only 61. Of this number only one developed clinical cholera and a second some manifestations of mild cholera. It is obvious that more healthy carriers exist in cholera than was previously supposed, and that these healthy contact carriers are important in the spread of the disease within the country and to neighboring and distant countries.

There is no animal reservoir host where the vibrio can survive in the inter-epidemic periods, but it is possible that in the endemic areas during these periods when the temperature and humidity are low, the vibrio survives in healthy carriers who transmit it from person to person without the production of gastrointestinal disturbances. These carriers are more likely to be infants and children than adults.

The chemotherapeutic sterilization of contact or convalescent carriers by absorbable or nonabsorbable sulfonamides could not be evaluated in the recent epidemic, as the majority of carriers became free of *V. cholerae* whether they were treated or not. However it is known that certain sulfonamides have a powerful vibriocidal action.

The fact, revealed by our laboratory tests, that *V. cholerae* are found in the intestinal tract in the vast majority of cases in the first 24 to 48, or possibly 72, hours of the disease is important in the specific treatment of cholera. As *V. cholerae* does not produce an exotoxin, it would seem that the symptom complex of cholera is produced by the endotoxin which is liberated after the death of the vibrio. According to Napier (9) "this endotoxin which is liberated within the intestine of the organism causes a superficial denudation of the epithelium of the intestine and increases its permeability so that there is a great outpouring of water and electrolytes with subsequent loss of fluids from the tissues and blood." He feels that the tissue changes are attributed to the absorption of this endotoxin as well as to the dehydration of the tissues themselves, hemoconcentration and low blood pressure which results from temporary ischemia. However there is no experimental evidence that the cholera endotoxin produces denudation of the epithelium of the bowel with increased permeability resulting in increased loss of electrolytes and water. It is likely that the clinical condition in cholera is one in which there is a combined action of a toxin and tissue anoxia and dehydration. The only possible explanation of the loss of water and electrolytes is the diarrhea and vomiting.

As *V. cholerae* is present in the stools only in the first few days of illness, any chemotherapeutic agent which is to be of value must be administered early in the clinical course of the disease. In cholera there is always the possibility of renal lesion, due to anoxia, the toxin, or both, so that the use of soluble, absorbable sulfonamides is out of the question. Thalamyd, a soluble, non-absorb-

able sulfonamide, has been found to exert a beneficial effect in cholera provided it is used early. If given later than the third day of the disease when the vibrio have usually disappeared from the intestinal tract its therapeutic value is lost.

SUMMARY AND CONCLUSIONS

1. A simple method for the laboratory diagnosis of cholera is presented: (a) Peptone water is inoculated with fresh stool and incubated 8 hours, (b) Alkaline nutrient agar plates are streaked and incubated overnight, (c) Transparent colonies are tested for agglutination with anti-cholera O serum. (d) Agglutinable vibrio are tested for hemolysis using 5 per cent sheep or goat cells.

2. *V. cholerae* are present in the intestinal tract only in the first few days of illness, therefore sulfonamides or other bactericidal agents are effective only if given early in the course of the disease.

3. The value of sulfonamides in cholera carriers cannot be definitely evaluated at this time.

4. Of 3,000 contacts, 61 became contact carriers. Two of these developed the disease. The number of clinical cases developing from contact carriers is very small.

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BRUCELLOSIS IN NORTH CHINA: A CLINICAL, ETIOLOGICAL AND EPIDEMIOLOGICAL STUDY¹

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Since the discovery in 1930, in the medical service of the Peiping Union Medical College Hospital (1), of patients suffering from naturally acquired brucella infection, we have maintained a continued interest in the subject and have attempted several types of study aiming to clarify especially the epidemiological aspects of this disease. First of all, the species of brucella producing disease in this locality, especially some of those that occurred among the technical staff of the Department of Bacteriology (2), has been determined. The occurrence of natural brucella agglutinin among a fairly large sample of population in Peiping has been observed (3) and compared with a similar study made in Shanghai where overt brucellosis is stated to be very rare (4). Finally, in 1941, a study of the infection among certain dairy cattle of Tientsin was also carried out (5). So far a systematic report of all the known cases of brucellosis seen in this city has not been made. In view of the absence of report of similar series of cases elsewhere in China, and in view of the recent interest in the distribution of various epidemic and endemic diseases all over the world as stimulated by global armed conflict (6), we have now analyzed the clinical material and studied the data so far obtained. At the same time, brief recapitulation is also made of the etiological and epidemiological studies previously made which seem to have a bearing on the subject under consideration.

CLINICAL STUDIES

From 1921 to the end of 1948, there were altogether 29 cases of proven and probable instances of brucellosis seen in the medical services of the Peiping Union Medical College and Chung Ho Hospital.³ Among these, six cases have been previously reported (1, 2) but are included for the sake of completeness. The clinical data may be presented in the following headings:

Contributory etiological factors: It is interesting to note that all the cases occurred in Chinese, with 18 males and 3 females among proven cases, and 7 males and 1 female among probable cases. The age and occupation of these cases are summarized in Tables 1 and 2.

As in most cases (7) brucellosis has involved most heavily young adult males, and our experience rather agreed with this general finding. The number is too small to show any preference for any specific profession except the medical personnel. For this group, a word of explanation seems to be in order. The 6 technicians were all workers in the Department of Bacteriology of the Peiping Union

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³ We are grateful to Dr. William H. L. Chung of the Chung Ho Hospital and members of his staff for permission to include their 7 cases in this report.

Medical College, and it is of interest to note that these came down in two batches, the first three, during May to September 1935, and the second three, November and December, 1937. In 1935 active bacteriological and immunological studies on *Brucella* were being carried out in the department, and it is quite conceivable that these technicians, among whom one actually helped in the immunization of the goat, could be so infected. However, during 1937, besides the

TABLE 1
Distribution of the age of patients with brucellosis

AGE IN DECADES	NUMBER OF PATIENTS	
	Proven	Probable
First.....	0	0
Second.....	6	3
Third.....	5	1
Fourth.....	5	1
Fifth plus.....	5	3
Total.....	21	8

TABLE 2
Distribution of occupation of patients with brucellosis

OCCUPATION	NUMBER OF PATIENTS	
	Proven	Probable
Medical Personnel		
Bacteriologists.....	2	0
Technicians.....	6	0
Nurse.....	0	1
Clerk.....	4	0
Students.....	2	2
Farmer.....	2	1
Peddler and Laborer.....	2	3
Butcher.....	1	0
Merchant.....	0	1
Total.....	21	8

routine carrying of stock cultures and occasional new isolations, no special study was made. None of the three technicians was directly connected with the isolation of fresh cultures. So the reason for the occurrence of infection among this latter group is not clear. Both the bacteriologists apparently contracted the disease after they have worked extensively with both *Br. abortus* and *Br. melitensis* cultures. How the nurse received the infection, it was not known.

Except for the case of the first bacteriologist, who came down with the disease in 1926, *Brucella* agglutinating antigen was routinely used beginning only from 1930. From then on, every year, there were a few clinical cases seen in the medi-

cal service of the Peiping Union Medical College Hospital. After the closure of that institution in February 1942, one of its senior visiting physicians, Dr. William H. L. Chung, reorganized the Chung Ho Hospital and carried on the established tradition. There were 7 additional cases recorded in the later institution

TABLE 3
Seasonal and yearly distribution of Brucellosis in Peiping

MONTH OF ONSET	NUMBER OF PATIENTS	
	Proven	Probable
January.....	0	1
February.....	1	1
March.....	4	1
April.....	1	1
May.....	4	1
June.....	2	0
July.....	1	2
August.....	1	0
September.....	3	0
October.....	0	0
November.....	2	1
December.....	2	0
Total.....	21	8
YEAR OF OCCURRENCE		
1926	1	0
1930	2	0
1931	2	0
1932	1	0
1933	4	0
1934	1	1
1935	3	0
1936	2	0
1937	4	0
1941	1	0
1943	0	3
1945	0	1
1946	0	2
1948	0	1
Total.....	21	8

from 1942 to 1948. The yearly distribution and seasonal distributions are presented in Table 3.

Symptoms and signs: Among the proven cases, 17 showed gradual and insidious onset, while 4 claimed to have sudden onset. On the other hand, 6 of the 8 probable cases were stated to have an abrupt beginning.

The initial symptoms were consistent in all cases of this small series. All com-

plained of irregular fever; most of these, 22, had chill; 12, sweating; 19, weakness; 13, marked anorexia or constipation; and the vast majority of cases, 22 out of 28, complained of pain in different parts of the body. Most of these symptoms persisted for a varying length of time, with fever, weakness and aching remaining the more prominent. As a rule, when the fever gradually lowered, most patients become more comfortable, their appetite gradually returned, and a number of them even gained weight in spite of the persistence of the low grade fever.

The only positive physical signs observed in this series were splenomegaly and hepatomegaly. The spleen was felt in all but 2 cases, while the liver was palpable in about one half of the cases. Both of these enlargements were mostly

TABLE 4
Types of fever curve in this series

TYPE OF FEVER	NO. OF PATIENTS	
	Proven	Probable
Undulant and relapsing.....	7	2
Intermittent and remittent.....	12	4
Atypical.....	3	2

TABLE 5
Duration of the fever

	PROVEN	PROBABLE
One-two months.....	4	4
3-5 months.....	10	2
6-7 months.....	5	1
1 year.....	1	1
Unknown.....	1	

minimal, but in certain cases, the spleen was felt down to 8 cm below the costal margin, and the liver, 6 cm.

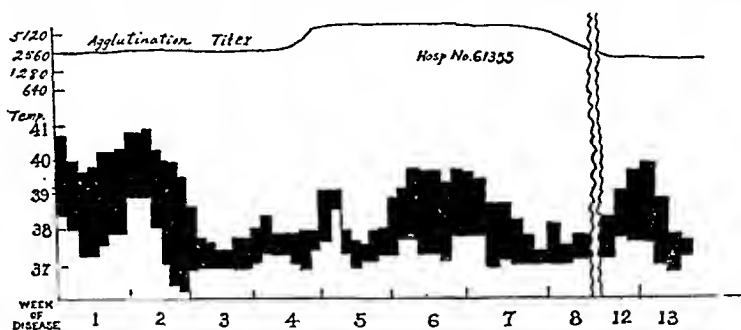
The temperature curve has received special attention in this disease because of its tendency to become relapsing and undulating, thus giving rise to the name, undulant fever. The temperature curve recorded for out patients may be classified in Tables 4 and 5.

It is of interest to note that the entire course of most of the proven cases lasted longer than 2 months, whereas at least half of the probable cases showed a shorter episode. It is possible when the disease was milder, positive blood culture was not so easily obtained, and at the same time, there was also less opportunity of securing specimens for repeated cultures. A few representative temperature curves are presented to show the various types actually observed.

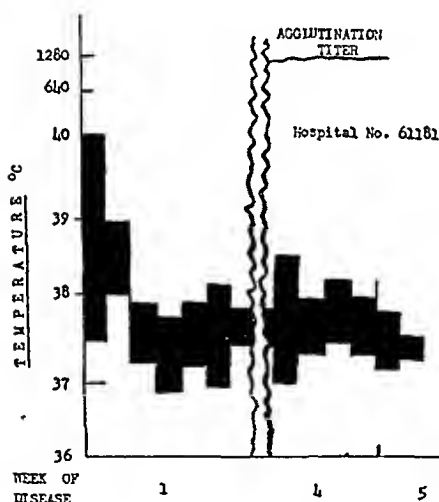
It is to be specially noted that we have a number of cases in the present series showing rather atypical fever. One such case lasting less than a month was proven by a positive isolation of the *Brucella* from the blood. Others were con-

sidered probable because of a definite and significant titre of positive *Brucella* agglutinin, several of them giving rising titre. These atypical cases may possibly be of real clinical and epidemiological importance because they might have served as the sources of infection and have kept the disease going on in this community.

Representative temperature charts of a severe and mild case of Brucellosis



(a) Severe and typical



(b) Mild

FIG. 1. (a, b)

Complications were extremely uncommon in this series. Aside from two cases with mild orchitis, and one with atypical pneumonia, the most important complications were bursitis in two cases and spondylitis in two cases. One of the latter was in fact admitted chiefly because of the chronic pain in the back together with the resulting weakness, and the diagnosis was only made by positive agglutination test and by positive culture from the synovial fluid. The details of this case are presented herewith.

Report of a case with spondylitis and bursitis: Peiping Union Medical College Hospital No. 40332, a professional donor aged 41, was admitted to the Medical Service of the Hospital on May 16, 1933 for a tender mass at the lower angle of the right mandible of four

days duration. Patient's previous health was excellent and his past illnesses irrelevant. There was no history of drinking raw milk of any kind and no contact with raw meat or close association with dairy animals. This patient was found to have a low grade fever when he was examined prior to a recent donation of blood. Following that, he became aware of the local tender swelling as well as headache and irregular fever. He was found to have acute osteomyelitis of the right mandible together with a cyst formed in the body of this bone. Spleen and liver were found to be slightly enlarged. His leucocyte count was only 3,500 per cmm. Blood cultures, were repeatedly found to be sterile, while the serum agglutination test against *Brucella* increased from negative up to 1:160 three weeks after onset. As the temperature came down gradually and pain disappeared, no definite diagnosis was made when patient was discharged from the Hospital. He was readmitted on September 4, 1933 for irregular fever, headache, and multiple joint pain for more than one month. In the meantime, the serum agglutination titre has risen to 1:640 as of August 23. Examination during second admission showed at one time bilateral acute serous olecranon bursitis; from the right side, *Brucella* organisms were cultured twice. Cultures made with blood and the synovial fluid from the left side were negative. He also complained of persistent pain in the lower back and repeated X-ray examination showed a spreading destructive osteomyelitis from the 3, 4, 5 lumbar vertebral bodies upwards to eventually involve the second also. Despite auto-vaccine and immobilization, it took the patient more than six months before the spinal lesion was considered quiescent. In the mean time, he ran from time to time irregular and low grade fever. Eventually the patient disappeared from observation and it was not known what finally happened to him.

This patient is reported in detail not only to add one more case of spondylitis complicating brucellosis (8, 9) but to record the occurrence of bilateral bursitis, from one side of which the etiological agent was recovered, while repeated blood specimens yielded negative results. Such localized infection is not unknown in animals infected with *Brucella*, but so far rarely reported from human cases (10). It probably indicates the advisability of employing more frequently materials from bone marrow, spleen or liver, for the cultivation of *Brucella* besides the routine blood cultures. Whether the osteomyelitis of the mandible was due to the same organisms, is impossible to say.

During the past twenty years, different methods for the treatment of the disease, from non-specific protein shock therapy, autogenous vaccine, immune serum, down through chemotherapy and even streptomycin, have all been used in our cases at different times. As the number of cases is far too few and the course of the disease so irregular, it is obviously not possible to state which therapy was beneficial. However, it is interesting that there was no death among our series, which also agreed with the published findings of low mortality in this disease as a whole.

Laboratory findings: As it is well known in brucellosis, a moderate anemia was universally found. Most of these cases also showed a fairly definite leucopenia during the height of the disease (11) which unfortunately does not help to differentiate it from typhoid, typhus, and kala-azar, the three most important acute and sub-acute fevers in this locality. The results of the white blood counts at different times of disease are presented in Table 6.

In most cases, repeated trials were made to culture different specimens of blood, urine, and feces specially for *Brucella* organisms. In a number of instances, Amoss and Poston's method of stool culture was followed (12), but always with

negative results. All the urine cultures were similarly negative, but it must be admitted that no special efforts were made to keep the cultures long enough and no inoculation into special medium was attempted. Special care was used in the making of blood cultures and the single fact which proved to be most important in this procedure is the duration of incubation of the culture for initial isolation. At least 10 days were allowed for the broth containing blood to be observed. With this precaution, we have found neither the special quality of the culture medium nor the use of carbon dioxide atmosphere was of importance in

TABLE 6
Total leucocyte count in brucellosis

CELL/CMH.	ACUTE STAGE				LATE STAGE	
	On admission		At fastigium		Proven	Probable
	Proven	Probable	Proven	Probable		
1-2,000	0	1	1	0		
2-3,000	4	0	6	0		
3-4,000	6	1	7	1	2	0
4-5,000	5	1	6	0	5	1
5-6,000	2	3			0	3
6-7,000	2	0			5	1
7-8,000					4	3
8-9,000	0	1	0	1	2	0
9-10,000						
10,000+	1	0	0	2	2	0

TABLE 7
Summary of bacteriological and serological studies

MONTH OF DISEASE	BLOOD CULTURE				AGGLUTINATION TEST			
	Proven		Probable		Proven		Probable	
	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.	Pos.	Neg.
1-2	19	7	0	3	25	4	5	1
3-5	15	8	0	2	26	0	2	0
6-13	2	9	—	—	7	0	3	0

increasing the number of positive cultures. In the proven cases, all except one gave positive blood culture at least once, whereas the only exception yielded a positive culture in the synovial fluid. All these cases similarly gave positive *Brucella* agglutination varying from 1:160 to more than 1:2560 at different time of this disease. The eight probable cases were diagnosed by means of either a very high agglutination titre, 1:1280, or 1:640, or an increasing titre, from negative, or from 1:40 to 1:320, in addition to the clinical findings. The results of repeated and monthly bacteriological and serological studies of these cases are summarized in Table 7.

As a rule, blood culture tended to be positive in the early active stage, while the agglutination reaction was practically never negative after the second month of disease. The latest positive blood cultures were found in the 6th month of disease while several patients showed negative cultures even during active stage of the disease.

ETIOLOGICAL STUDIES

All the organisms isolated in blood culture were subjected to agglutination study with specific antiserum prepared in this laboratory with standard cultures. All except one gave definite and conclusive serological evidence. The last one was diagnosed from cultural characteristic alone. One of these on primary isolation behaved like *Brucella para-melitensis* because of its spontaneous agglutinability in normal saline even at 37°C., and was so diagnosed. Five of the other cultures were subjected to special studies, such as growth in media con-

TABLE 8
Special studies on five strains of Brucella

	H ₂ S prod.	FLOCCULATION AT 90°C.	INHIBITION BY		AGGLUTININ	
			Thionin	Basic fuchsin	Absorption by <i>Brucella</i>	
					melitensis	abortus
<i>Br. melitensis</i>	±	—	+	0	complete	incomplete
<i>Br. abortus</i>	+++	—	±	±	incomplete	complete
No. 29474	±	—			complete	complete
No. 31599	±	+			incomplete	incomplete
No. 44788	+	—	±	±	complete	incomplete
No. 51283	+	—	+	0	complete	incomplete
No. 22857	++	+	+	0	none	none

taining dyes, H₂S production and agglutinin absorption test (13). The results are summarized in Table 8.

From the above, it seems that 3 of the 5 local strains studied probably were *Brucella melitensis*, and from the results of absence of requirement for CO₂ during initial isolation, it seemed our previous conclusions (1, 2) that most, if not all, our strains locally isolated were either *Br. melitensis* or *Br. para-meli-tensis*, was justified.

EPIDEMIOLOGICAL STUDY

In view of the recurrent incidence of local patients affected with *Brucella* since the clinical bacteriological laboratory initiated the agglutination test with *Brucella abortus* in 1930, an inquiry has been made into the distribution of the possible latent infection as well as carriers among the local population (3), and a preliminary study of the *Brucella* infection among dairy cattle in North China (5). More than 2000 specimens of blood from normal persons and patients with a variety of diseases were examined for the presence of *Brucella* agglutinin. It

was found that more than 8 per cent of the specimen gave a positive agglutination at 1:10 dilution, 3.2 per cent, at 1:20, 0.8 per cent at 1:40 and 0.2 per cent at 1:80 and 1:160. All of these were found to be in the age group above 5 years old and a gradual increase in positiveness could be noted up to adult life. These results when compared with similar studies elsewhere seem to suggest that *Brucella* infection may be endemic in Peiping. On the other hand, among the Chinese in Shanghai, where the only similar study was made, and where overt *Brucella* infection is rare (4), only 1.9 per cent showed positive agglutinin at 1:10, and 0.9 per cent at 1:20. None showed agglutination titre at 1:80 or above.

Examination of a few cattle in three dairy herds in Tientsin revealed the interesting findings that in the first place, many of the aborted cows had a high agglutination titre to *Brucella* antigen. Although no organisms were isolated from these cattle, by applying absorption test (13) to the sera, it was found that these animals seemed to be infected with *melitensis* rather than *abortus*. That such a condition is possible has recently been repeatedly confirmed else-

TABLE 9

Contact history of Brucella cases

1. Contact: with sheep 2 (1 case 20 years ago) with goat 1
2. Meat: butcher, 1; neighbor to meat shop and slaughter house, 2
3. Milk: drank boiled goat milk, 3; boiled goat milk, 3 (one case gave such a history one month before onset)

TABLE 10

Analysis of possible laboratory infection

- | | |
|-----------------------------|-------------------------------------|
| 1. Definite exposure known: | 2 bacteriologists, 1 technician |
| 2. Doubtful exposure: | 2 technicians, 2 laboratory helpers |
| 3. Probable no contact: | 1 serological technician, 1 nurse. |

where (14, 15, 16, 17). Unfortunately, the contact history of our patients does not yield any definite information. Although special emphasis was paid to this point in inquiring our patients, the only information obtained is in Table 9.

In view of the high percentage of cases occurring among medical personnel, the accompanying analysis is made in Table 10.

DISCUSSION

Sixteen years after the discovery of the etiological agent by the British Commission, Manson (18) already made the following statement: "We have seen cases in China having all the clinical symptoms of Malta Fever." Boone (19) however was the first to record 2 cases of human infection in China, one of whom was a foreigner, the other, the nationality was not definitely stated. In the course of the next year, another report of 3 cases in Chungkiang appeared in the China Medical Journal (20). At the same time it was stated in the report that these were the first instances noted in the last 15 years. Later Maxwell (21) reported a case from Fukien, and Rohow (22) reported one from among the American Marines in Shanghai. All these were diagnosed on clinical and/or

serological grounds. It remained for Lim (23) in 1925 to make the earliest report in China of bacteriologically proven instances, all of whom were among the foreign population. The first isolation was made among a member of Sikh Watchmen imported together with their goats, from the Punjab to Chiao-tso mines in Honan. The second instance involved a Russian from Harbin. Both these cultures were identified as *Brucella melitensis*.

Since our report in 1932 (1) of bacteriologically proven cases among the Chinese patients in Peiping, few additional ones have been reported in the literature outside of Peiping (4, 24). On the other hand, *Brucella* infection among the dairy animals in China has been reported from time to time. Gear (25) in a report to the Ninth Congress of the Far Eastern Association of Tropical Medicine had this to say: "It has been reported from the Sanitary Department of Hong-kong (that), occasional cases of contagious abortion thought to be due to brucellosis were observed as early as 1910, and it was also known to have occurred among dairy cattle in Shanghai." In Nanking, the following data were obtained in 1928 by Gibbs (26):

Foreign imported cattle.....	29.6 per cent positive agglutination
Chinese yellow cows.....	9.0 per cent positive agglutination
Water buffalos.....	4.7 per cent positive agglutination

Besides the small series reported by one of us (5), two reports have recently appeared on the incidence of *Brucella* infection among the dairy herds; one was by Sheng of the Northwest Epidemic Prevention Bureau in Lanchow (27) on infected cattle in the northwest China, while another by Hung (28) extended the previous observation of Japanese workers (24) on the incidence among sheep and cattle in Manchuria. Both of these investigations made use of the serological reaction. It might be noted that in the *Brucella* cultures collected by Huddleson (29) in the Central Brucella Station, Michigan State College from 1920 to 1942, a strain of *Br. abortus* was recorded to have been received from China said to have been isolated from the cow.

Thus it seems that *Brucella* infection in China, at least among the animals, cannot be considered as uncommon as writers on tropical medicine would lead one to believe (6). The infrequent discovery of this disease among Chinese might be explained by the possible existence of mild cases as exemplified by several of our patients, as well as by the studies of Shiroki (24) which were only proven by isolation of the causative organisms from the blood culture or by positive serological evidence. It may partly be due to the rather infrequent routine use of *Brucella* agglutination test throughout the country.

From the serological study of the blood serum of apparently infected cattle, and the cultural and serological study of the *Brucella* organisms isolated from patients, it is wondered if one may postulate the following course of events. *Brucella melitensis* infection may have been introduced into China through infected goats (23), and since then they have either directly been transmitted into the cattle, or indirectly through patients and then to the cattle. For the former possibility, we have precedents as reported in other countries (17). The

latter is merely a suggestion in view of the close association of human beings with domestic and dairy animals among a large section of the population in China, and in view of the possible existence of a number of carriers as is evidenced by high percentage of positive *Brucella* agglutinin in the local population, the transmission of organisms from men to animals may not be considered unlikely.

It is interesting to recall that as early as 1931, Rohow (20) suggested that his case was one due to *Brucella melitensis* from direct serological evidence. He also suggested that danger from drinking goat milk as well as from other sources of infection exists in China. He further believed that such cases are not rare but are not diagnosed because of lack of facilities. His case, too, was mild, the total disability lasting only 61 days.

SUMMARY

From 1930 to 1948, when *Brucella* agglutination test was routinely used in Peiping Hospitals for the diagnosis of unknown fever, there were observed 20 bacteriologically proven cases. Besides, another proven case had occurred in 1926 when a bacteriologist contracted this disease, probably through contact with the cultures. There were 8 additional instances which were included in the present report because of the likely clinical findings plus definitely positive serological evidence. The important clinical features and the results of laboratory examinations of these cases were summarized. At the same time, etiological and epidemiological studies previously conducted are summarized and their significance briefly discussed. A case with spondylitis and bilateral olecranon bursitis was reported *in extenso* because of its apparent unusual occurrence.

ACKNOWLEDGEMENT

We wish to record our indebtedness to members of the clinical departments of the Peiping Union Medical College Hospital who took great interest in the clinical studies of these cases, without whose efforts, this report would not have been possible.

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STUDIES ON THE EMERGENCE OF CERCARIAE OF *SCHISTOSOMA*
JAPONICUM FROM THEIR CHINESE SNAIL HOST,
*ONCOMELANIA HUPENSIS*¹

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INTRODUCTION

Studies on the emergence of cercariae from their snail host are rather scarce. Cort (1922), while working with *Cercaria elephantis*, first pointed out the possibility of the influence of temperature on the number of cercariae shed. Horsfall (1930) considered the same factor to be important in the shedding of *Cercaria infracaudata*. Rees (1931) observed a favorable influence of light and increase of temperature on the shedding of *Cercaria cambrensis* I. The effect of light was demonstrated beyond doubt by Giovannola (1936) who artificially reversed the periodicity of emergence of cercariae by reversing the daily light cycle. Rees (1948) demonstrated that under laboratory conditions the discharge of *Cercaria purpurae* was influenced by light, temperature and salinity.

Concerning the cercariae of *Schistosoma mansoni*, Faust and Hoffman (1934) were the first to point out that the cercariae escape from their snail host between 9 a.m. and 2 p.m. Gordon *et al.* (1934) reported that transference of snails from the refrigerator to room temperature or incubator temperature stimulated the release of cercariae. The same effect was obtained when the snails were exposed to sunlight but the authors were inclined to believe that light was not as important as temperature in determining the time of shedding of the cercariae. This was demonstrated in another way by Kuntz (1946) who stated that "under bright light and elevated temperatures cercariae can be forced to emerge from infected snails either three times during the same day or in moderate quantities daily for a period of five to nine days."

Very few studies have been made on the emergence of *Schistosoma japonicum* cercariae. Isobe (1923), working in Formosa, found that the time required for the cercariae to emerge from the snail, *Oncomelania formosana* (= *Blanfordia formosana*) was as follows: 4-5 hours at 18°-19°C., 3½ hours at 21°C. and 2 hours at 24°C. No shedding was observed at temperatures below 15°C. The nature of the water used made no marked difference. According to Isobe, light did not stimulate shedding and darkness was more favorable for shedding than light. Osaka (1938) reported that (1) the most important influence upon the escape of *S. japonicum* cercariae from the snail vector in Japan was the water temperature while neither light nor the period of dryness played any significant role; (2) the time used for the cercariae to escape from their snail host depended upon the temperature of the water, *i.e.*, the warmer the water, the shorter the time; (3) the cercariae do not escape from their snail host continuously but alternate long periods of shedding activity with shorter periods of rest; (4) the period during

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which cercariae escaped varied from $1\frac{1}{4}$ hours to $5\frac{3}{4}$ hours. Bauman *et al.* (1948), working with infections in *Oncomelania quadrasi* in the Philippines, concluded that the natural release of cercariae occurred at night. Their experiments also showed that "those snails exposed to bright illumination released considerably fewer cercariae than did the control group (without artificial light)." Yet, they added further, "light or darkness had little effect, if any, on the escape of cercariae." Temperatures, within the range of 19°–30°C. seemed equally suitable for shedding; on the other hand, the pH of the water was considered to exert a great influence on shedding as evidenced by "a marked increase in cercarial emergence" in river water with a pH of 7.6 and the fact that "some cercariae were released in two controls with pH 7.2 whereas none were recovered in control tests of pH 6.4, 6.6, 6.8 and 7.0." A cyclic production of cercariae was suggested by the same authors, based on the fact that after 2 or 3 consecutive isolations "an equally long period would pass before any considerable number of cercariae would emerge."

In view of the apparent differences in the findings just noted we decided to attempt a further study of the factors influencing the emergence of *S. japonicum* cercariae from the Chinese snail host, *Oncomelania hupensis*.

TECHNIQUE

Owing to the presence of the operculum and the amphibious habits of the snails, obtaining accurate counts of *S. japonicum* cercariae shed by individual snails offers special difficulties not met with in dealing with other schistosome species. After several trials we decided to use flat bottomed test tubes, 10 x 100 mm., for the snail isolations. They were kept together in groups of 30–40 by rubber bands, each group in a petri dish of 10 cm. diameter. The tubes were filled almost full with water, one snail was added to each tube, and the bundle then covered with a square glass plate. Cercaria counts were made by placing the petri dish on a ground glass which formed the cover of a wooden box containing two 40-Watt electric bulbs and examining the surface of each tube with a 15x hand lens. This was usually done after removal of the glass cover but when water adhered to the plate the cercaria counts were made before its removal. The illuminating box supplied both light and heat, the latter could be controlled by inserting one or several glass plates between the ground glass cover and the dishes. Temperatures in the tubes were determined by placing a thermometer in one of the tubes in the group that had no snail. Unless otherwise indicated, the water used was filtered river water from Chi-hsia-shan, a newly discovered endemic focus of schistosomiasis japonica about 20 kilometers northeast of Nan-king. All glassware and instruments were regularly sterilized by boiling. Between tests the snails were kept in moist, unglazed earthen jars or dishes and fed with Chinese cabbage, *Brassica chinensis*. The details of rearing and feeding of the snails will appear in a separate paper. All the snails, with the exception of those used in the first experiment that follows, were positives isolated by shedding tests of snails collected in Chi-hsia-shan or in Soochow. In spite of some minor morphological differences in the shells of the snails from the two areas, we consider

them all *O. hupensis* for reasons which have been mentioned elsewhere (Mao and Li, 1948).

RESULTS

Influence of temperature on the shedding of S. japonicum cercariae: It was first decided to find out whether there was an optimal temperature at which all of the infected snails would shed cercariae. Snails were tested within 40 hours after being collected from the wild. The number of snails used in each test varied from 30 to 233 and a total of 35 tests were conducted between Oct. 20 and Dec. 4, 1948. Each experiment was started at 9–10 a.m. and readings were made four hours later. Positive snails were picked out and the remaining snails were dissected and all harboring mature infections were counted. The ratio of those which

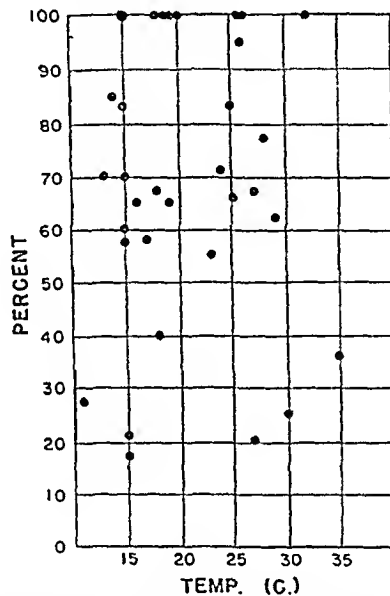


FIG. 1. PERCENTAGES OF SNAILS SHEDDING CERCARIAE IN 35 EXPERIMENTS DONE AT TEMPERATURES BETWEEN 11° AND 35°C.

shed to the total infected then provided the percentage of infected snails which shed in each experiment. Of 3,209 snails tested, 218 were found to be positive by the shedding method while an additional 111 were found by dissection. The percentage of infected snails which shed in each of the 35 experiments is presented in Figure 1. From the results of these experiments it is evident that within the range of 11° to 35°C. the proportion of snails shedding cercariae did not vary with the temperature.

To determine whether there is a quantitative difference in the number of cercariae shed at different temperatures, a second series of experiments was carried out. Ninety-six infected snails were divided into 3 groups. Group I was placed in an incubator at 35°C., Group II was held at room temperature (19.5°–21°C.) and Group III was kept in an ice chest at 5°C. As it was completely dark in the incubator and ice chest, Group II was kept in total darkness also. The

tubes were set up at 8 a.m. and cercaria counts were made at one hour intervals until 4 p.m. The snails were then taken out of the tubes, mixed and returned to the earthen vessels. The next day they were again divided into 3 groups and the experiment repeated as before. Five days later a third test with these snails was made in the same way. The combined results of these three tests are shown in Figure 2. The percentage of snails shedding more than 50 cercariae did not differ appreciably in the two groups at 20° and 35°C. At 5°C. none of the snails shed over 50 cercariae.

Influence of light on the shedding of S. japonicum cercariae: Seventy-four infected snails were divided into two groups. Group I was kept under artificial light supplied by a 40-watt bulb and maintained at a temperature of 14°C. Group II was kept in the dark at 13°C. The tubes were set up at 8 a.m. and the

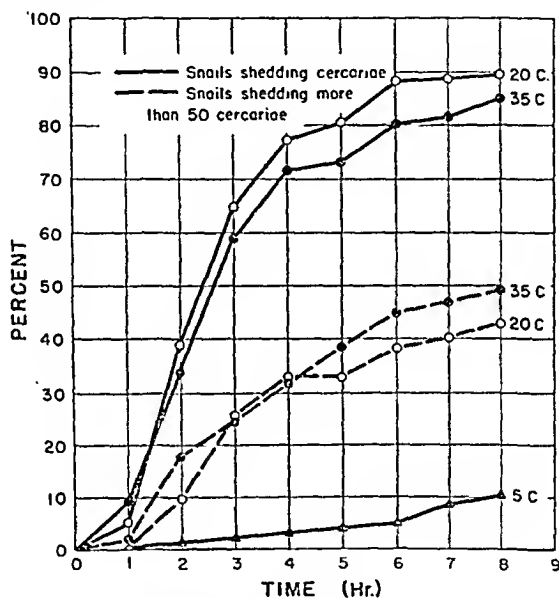


FIG. 2. PERCENTAGES OF SNAILS SHEDDING CERCARIAE AT 5°, 20°, AND 35°C. IN RELATION TO THE LENGTH OF TIME THE SNAILS WERE IMMERSSED IN THE WATER

number of cercariae discharged was counted at 4 p.m. The snails were then taken out of the tubes, mixed, fed and three days later redivided and retested in the same way. In the second test the temperature under artificial light was 17°C. and that in the dark 15°C. The results of the two tests are shown in Sections A and B of Table 1.

A similar experiment was then performed using higher temperatures. Thirty-seven snails were put in an incubator at 26°C. in complete darkness, and another 37 on the illuminating box at a temperature of 25°-27°C. The tubes were set up from 9 a.m. to 3 p.m. The results are shown in Section C, Table 1. It appears that in these tests light had a marked influence on the emergence of *S. japonicum* cercariae.

Since our findings differed so much from those of previous workers, we decided to recheck our results by carrying the experiment further. Thirty-seven infected snails that had been kept in the dark in the previous experiment were now put

TABLE 1

Emergence of Schistosoma japonicum cercariae under various conditions

	TEMPERATURE	TIME	NUMBER SNAILS TESTED	NUMBER SNAILS SHEDDING	NUMBER OF CERCARIAE SHED BY A SINGLE SNAIL		
					Maximum	Minimum	Average
	°C.						
<i>Section A</i>							
Light	14	8 hrs.	37	15	306	1	121
Darkness	13	8 hrs.	37	1			21
<i>Section B</i>							
Light	17	7 hrs.	37	21	600	14	227
Darkness	15	7 hrs.	37	4	54	1	16
<i>Section C</i>							
Light	25-27	6 hrs.	37	21	610	2	170
Darkness	26	6 hrs.	37*	8	13	1	4
Light		1 hr.		17	304	1	66
Darkness		1½ hrs.		17	15	1	5
Light		¾ hrs.		20	68	1	21
<i>Section D</i>							
Light	32	7 hrs.	37	24	970	2	51
Darkness	15	7 hrs.	37	1			5
<i>Section E</i>							
Light	14	4 hrs.	37	10	293	2	78
Darkness	32	4 hrs.	37	3	4	1	3
<i>Section F</i>							
River water	22	7 hrs.	74	53	1,650	1	398
Tap water	22	7 hrs.	37	21	1,240	4	290
<i>Section G</i>							
River W. pH 7.2	22	8 hrs.	18	12	934	1	201
River W. pH 7.8	22	8 hrs.	18	13	1,008	1	254
Dist. W. pH 6.6	22	8 hrs.	18	1			2
<i>Section H</i>							
River W. pH 6.6	22	7 hrs.	17	12	564	3	132
Dist. W. pH 7.8	22	7 hrs.	17	2	42	6	20
<i>Section I</i>							
Moist	22	1 hr.	40	0			
		2 hrs.		2	203	95	199
		3 hrs.		14	327	1	78
		4 hrs.		17	465	1	137
Dry	22	1 hr.	40	0			
		2 hrs.		0			
		3 hrs.		9	60	1	14
		4 hrs.		14	190	1	45

* The same snails were immediately used for the tests that followed in the same experiment.

on the illuminating box from 3:30 to 4:30 p.m. Cercaria counts were made and the snails were then transferred to fresh tubes. These were kept in the dark from 4:45 to 6:15 p.m. After the number of cercariae shed was again recorded the same snails were again changed to fresh tubes and put on the illuminating box from 6:30 to 7:15 p.m. The results of this last series of observations, recorded in Section C of Table 1, indicate further that the emergence of the cercariae is definitely influenced by light.

The combined effect of light and high temperature was then tested. The results of this test are shown in Section D, Table 1. They indicate that snails kept illuminated and at a high temperature shed more successfully than those kept dark and at a lower temperature.

In another experiment one group of infected snails was kept in a warm and dark place and another group in a cool lighted place. The results are tabulated in Section E, Table 1. In this test more cercariae were shed by the second group of snails.

Influence of pH and nature of the water on the shedding of S. japonicum cercariae: First we compared river water with tap water having pH values of 7.2 and 7.3 respectively. Seventy-four snails were used for the river water and 37 for the tap water. The number of snails shedding cercariae as well as the number of cercariae shed were recorded at one-hour intervals for 7 successive hours. The results are tabulated in Section F, Table 1. The differences between the two groups of snails are probably not significant.

A second test was designed to compare the emergence of cercariae in waters having various pH values. Fifty-four infected snails were divided into 3 equal groups. One was placed in distilled water of pH 6.6, another in river water of pH 7.2, and the third in river water alkalized to pH 7.8 with NaOH. All the pH values were determined by Hellige comparator. The tubes were set up at 9 a.m. and illuminated uniformly. Cercaria counts were made at two-hour intervals until 5 p.m. The results are presented in Section G, Table 1.

As it is difficult to tell whether the failure of cercariae to emerge from the snails kept in distilled water was due to the acidity of the water or to other characteristics of the water, a third test was carried out as follows: Seventeen infected snails were put in tubes containing river water acidified to pH 6.6 and another 17 in distilled water alkalized to pH 7.8. The results are shown in Section H, Table 1. Since the shed was good in the river water at pH 6.6, it seems probable that other characteristics than the acidity of the water inhibited the shed in the distilled water in the previous test. It was noted in both tests that most of the snails put in distilled water remained in the bottom of the tube with the body retracted. All were able to resume their activity once changed into river water.

Influence of dryness and moisture on the shedding of S. japonicum cercariae: We had presumed that perhaps cercariae would emerge more readily from snails that had been kept dry for some time. To test this assumption, 80 infected snails were divided into 2 equal groups and placed in unglazed earthen jars. One jar was kept dry while the other was put in a large dish of water which soaked the

jar and kept its inside walls moist. The snails were kept in these conditions at room temperature (10°–15° C.) for 17 days and during that period it was noted that the snails in the dry jar remained quiescent, with their opercula tightly closed while those in the moist jar were quite active. To test them for shedding, the snails were taken out of their respective containers and put in tubes in the ordinary way. Readings were made at one-hour intervals for 4 hours. The cercaria counts are recorded in Section I, Table 1. It seems from these results that snails kept moist shed more readily than dry ones. Not only was the average number of cercariae greater, but the time required for the cercariae to begin to escape was also shorter.

DISCUSSION

We find several discrepancies between our findings, just recorded, and those reported by other authors. In the first place, Isobe (1923) and Osaka (1938) have considered that temperature is an important factor in the shedding of *S. japonicum* cercariae. Bauman *et al.* (1948) found no significant difference in shedding at temperatures between 19° and 30°C. Under our experimental conditions, cercariae were shed just as well at 15°C. as at 35°C. Small numbers of cercariae were observed to escape from some snails even at 5°C. It is concluded that within the range of temperatures ordinarily encountered in the warmer seasons of the year, temperature is not a prime factor in determining the shedding cycle of *S. japonicum* in China.

The influence of light on the shedding of *S. japonicum* cercariae has been discredited by previous workers, yet our experience indicated that it can play an important role, at least when one is dealing with infections in the Chinese snail vector. From our findings it seems possible to conclude that, in China, under natural conditions, the shedding of *S. japonicum* cercariae takes place during the day time. This is at variance with the findings of Bauman *et al.* in the Philippines who observed a nocturnal periodicity, not due to the absence of light. Cram (1947) noted that there was a difference in the shedding characteristics of *S. japonicum* from *Oncomelania quadrasi* as compared with infections in the other two host species. There might be strain differences here to account for the divergent shedding results.

Bauman *et al.* (1948) found that pH and the nature of the water used were of great importance in regulating the shedding. Yet our findings indicate that within the pH range of 6.6 to 7.8, cercariae emerge equally well from the snail host. We have shown, furthermore, that slight acidity (pH 6.6) was not the factor that made distilled water an unfavorable medium for shedding.

Although Osaka (1938) stated that a period of dryness stimulated a good shed of cercariae when the snails were put back into the water, in our experience snails that were kept moist shed more successfully when put into water than did those that were kept dry.

SUMMARY AND CONCLUSIONS

1. Light plays an important role in the emergence of *Schistosoma japonicum* cercariae from their Chinese snail host, *Oncomelania hupensis*.

2. Temperature plays a less important role since in our experience no quantitative difference was observed between 15° and 35°C. Shedding from some snails occurred at 5°C. but the number of cercariae shed was small.

3. Water with pH values ranging from 6.6 to 7.8 seemed equally suitable for the shedding of *S. japonicum* cercariae.

4. Tap water and filtered river water were both suitable for shedding of the cercariae but distilled water was unfavorable.

5. Infected snails kept moist showed a greater readiness to shed cercariae than those kept dry.

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THE DIAGNOSIS AND PROGNOSIS OF SCHISTOSOMIASIS¹

MICHAEL GELFAND²

Schistosomiasis can be recognised in the majority of instances, although in many the demonstration of eggs is not easy. The diagnosis rests ultimately on the finding of ova or of certain conclusive or highly suggestive signs of the disease.

Diagnostic criteria, such as the intradermal reaction, the blood eosinophilia and the formol gel test, occupy a useful place in its recognition. Nevertheless, it is my experience that they are of limited value and their results in any particular case should only be accepted after careful consideration of the clinical data available.

The blood eosinophilia is a pointer to the presence of the disease but its absence should not exclude it. In my experience, about 50 per cent of bilharzial cases do not show an eosinophilia. Further, even when it is present it may be due to other causes.

The intradermal skin test, if positive, is a factor pointing to the presence of the disease. Should it be negative, on the other hand, the patient may still harbor the infection. In a recent series of 100 Africans passing ova the skin test was negative in 34. *Vice versa*, positive reactions were obtained in about 20 per cent of a series of non-bilharzial subjects. In the latter series, a positive reaction was taken as a definite increase in the size of the weal. The fact that it is difficult to define a positive reaction is, I feel, the chief drawback to the test. What increase in size of the weal is to be accepted as constituting a positive reaction? For instance, Fairley and Williams (1927) laid down that a positive reaction should be at least 1 inch in diameter, whereas Most and his colleagues (1947) state that an increase of 4 mm. is sufficient.

There need be no serious controversy over the skin test. Perhaps the medical practitioners in the Colony have read more into the test than has been claimed by those who introduced it. In no publication by Alves and Blair (1946 and 1947) is there any statement that a positive skin test means that the patient is suffering from bilharziasis. In other words, they did not claim that the test is absolutely specific and that it should replace a thorough effort for the actual demonstration of the ova.

There are three reasons why the skin test cannot be regarded as a specific test for the disease:—

- (1) A positive reaction may occur in those not affected by the disease.
 - (2) A negative reactor may yet have the disease.
 - (3) There is still no unanimity as to what constitutes a positive reaction.
- Of course this does not mean that the skin test has no place or value in the diag-

¹ This paper is published with the kind permission of Dr. R. M. Morris, Secretary for Public Health, Southern Rhodesia Government.

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nosis of the disease. It plays a rôle just as the blood eosinophilia and the formol gel tests do.

It is claimed that from time to time, one may encounter cases in which ova are deposited in viscera other than the bladder or rectum and hence are not found. My post-mortem experience so far tends to show that such ectopic deposits are extremely uncommon and probably constitute no more than 1 per cent to 2 per cent of patients. It is in this very small group of cases where, if there is a clear history of exposure together with highly suggestive constitutional symptoms and a positive result in one of these tests, it may be considered worth while to institute a course of antimony.

I have pointed out that the sole criterion of the diagnosis of the disease lies in the demonstration of ova, which, as already stated, can be found in the vast majority of cases. It is now my task to discuss the methods by which these eggs may be found.

The first procedure obviously lies in examining the urine or stool. The examination of a single specimen, as you are all aware, is often unsuccessful. Consequently several samples should be submitted to the laboratory for such investigation. Ogier-Ward (1945) finds that five consecutive early morning specimens will successfully reveal ova or red cells in 85 per cent of cases. Nevertheless it is our experience here that, in spite of a very careful search in many specimens, it is not always possible to find the ova by simple urine and stool examination. The reason for the difficulty of demonstrating ova in these cases probably depends on the egg-lying capacity of the female schistosome, since I have noticed at the Native Hospital that a patient with *S. haematobium* may, at different intervals, pass urine which is perfectly clear on one day and yet contain albumin, red blood corpuscles and ova on another occasion.

I have found that the diagnosis of the disease in a patient suffering from *S. mansoni* can be more readily established than in one with *S. haematobium*, although it is usually taught that *S. mansoni* infestations are much more difficult to recognize. It is not that a stool is easier to examine than a specimen of urine, but, from my experience in the Native Hospital, the figures for those patients in whom both stools and urines were investigated reveal that *S. mansoni* was more frequently found than *S. haematobium*. At autopsy, however, *S. haematobium* is very much more often present than *S. mansoni*. As an individual passes only one or two stools a day, the ova are more likely to be collected in the faecal mass than in each of the many samples of urine voided over the same period.

Before 1943, the recognition of the disease in those cases in whom ova could not be found by microscopical examination of the excreta, was unsatisfactory. In approximately 20 per cent to 40 per cent of cases, the diagnosis could not be established by this usual method. In this year Ottolina and Attencio, two workers from South America, showed first at autopsy and later clinically that the ova of *S. mansoni* are deposited diffusely and evenly throughout the rectal mucosa. In other words, if a small portion or snip be removed from any part of the rectal mucosa, lateral-spined ova would be found. They claimed, in fact, 100 per cent results. This was confirmed by other South American workers, notably Hernandez-Morales and Maldonado (1946).

Employing this technique in Southern Rhodesia (1947) I found that about 60 per cent to 70 per cent of cases, both at autopsy and in those passing ova in the stool, yielded positive snips. My results were not so high as those obtained elsewhere. On the other hand, a number of cases with negative stools revealed positive rectal snippings. Thus by the combination of stool examination and the rectal biopsy technique, about 85 per cent of cases with intestinal schistosomiasis can be recognized.

An advantage of the rectal biopsy technique in this Colony is that urinary schistosomiasis may sometimes be diagnosed by it. I have noticed that the rectal snippings of about 40 per cent of sufferers from urinary bilharziasis reveal ova of *S. haematobium*. Although, in my experience, it is rare for the eggs of *S. haematobium* to be excreted in the faeces, yet at autopsy, when the bladder and rectum were each separately digested in 200 bilharzial subjects, no less than 73 per cent of the rectums contained terminal-spined ova. Further, snippings taken at autopsy in the rectum prior to digestion not uncommonly showed these ova either alone or in combination with *S. mansoni*. Thus, by taking a rectal snip, not only can one find the ova in the majority of *S. mansoni* infections, but also in about 40 per cent of the urinary ones.

The diagnosis of urinary schistosomiasis at present, rests with an attempt to find the ova in the urine by repeated examination of specimens or by cystoscopy. All writers on this disease stress the importance of the bladder changes recognizable cystoscopically in every case. Thus if ova cannot be demonstrated in the urine, then the teaching is that cystoscopy will determine whether or not the disease is present. When I first studied this problem at autopsy, I presumed that if the bladder appeared normal to the naked eye, the disease could be excluded. However, when each bladder at necropsy was digested routinely, whether it appeared normal or not, I found that in 30 per cent of those showing ova on digestion no naked eye lesions could be seen. In other words, a normal-looking bladder does not mean that a patient is not suffering from the disease, although in about 70 per cent of cases gross changes are obvious.

As with the rectal biopsy technique, if snippings are taken from any portion of the bladder mucosa, ova will be found in a very high percentage of cases—80 per cent to 90 per cent (Gelfand, 1947). Thus by employing the cystoscope, together with the snipper, one should be able to diagnose *S. haematobium* infestations in the great majority of cases.

I wish now to refer briefly to a diagnostic sign which I am finding often enough in the Salisbury Native Hospital to be of value, although limited, in the diagnosis of the disease. It is calcification of the bladder, as seen radiologically. This sign has been recognized for a number of years in Egypt, but except for scant mention in books, does not appear to have received much attention or been employed to any great extent. I find that when a straight radiogram of the bladder region is taken in a subject with urinary bilharziasis, about 10 per cent to 15 per cent show a thin, clear, curved line of calcification of varying length (Figs. 1, 2 and 3). In some cases the line is almost a complete circle and may, in fact, resemble a foetal skull. In others, it is very short, narrow and faint. Between these extremes there are all gradations. There is little else I know that can stimulate the radio-

logical appearance of a calcified bladder. In African practice, I have found this sign useful and simple to demonstrate. Provided the equipment is available, this procedure is quick and it causes no inconvenience to the patient. In this respect it is preferable to biopsy snippings or cystoscopy. A serious disadvantage of this sign, however, is that it is rarely seen in European practice, probably because the native, on the whole, has the infestation for a longer period and more severely than does the white man.

This brings me to the second half of my paper, which deals with the prognosis or outlook in schistosomiasis. Is it a serious disease as regards life and how grave are the lesions in any particular organ? The answer to these questions



FIG. 1. CLEAR THIN LINE OF CALCIFICATION ON INFERIOR AND LATERAL ASPECTS OF BLADDER

cannot as yet be stated with any authority, but my findings at autopsy have led to certain deductions which, if proved correct, may be of assistance in assessing the gravity of the disease.

A study of the literature will at once convey the serious view taken by the profession. The writings from Egypt, the Far East and South America all stress the debilitating and often life-destroying effects of both forms of the disease.

In this paper I particularly wish to refer to the sequelae of the disease which are regarded as grave or liable at any time to lead to fatal complications. What the exact morbidity is I shall not discuss here, except to refer to the incidence of the disease in Mashonaland. The more bilharzial subjects there are, the greater the sickness rate must be.

The true incidence, in my opinion, can only be accurately determined at autopsy. By digesting the entire bladder and rectum of each subject in caustic potash and examining for ova, a very good idea of the frequency of the disease must be obtained. In my series of 400 adult African autopsies, the incidence was 90 per cent. I doubt whether there is any other area in the world with such a high incidence. It is now necessary for me to mention briefly the effects of the disease generally regarded as serious. The organs chiefly considered to be gravely affected are the liver, appendix, lung, ureter and bladder.

With regard to the liver, most workers in the field today agree that schistosomiasis may be complicated by hepatic cirrhosis. The liver changes are well



FIG. 2. ALMOST COMPLETE CIRCLE OF CALCIFICATION WITH IRREGULAR CALCIFIED ISLANDS IN THE CENTER OF THE BLADDER

known to you and include a portal cirrhosis indistinguishable from Laennec's cirrhosis, *i.e.*, portal obstruction with ascites. Haematemesis or a cholaemia may eventually supervene to carry off the patient. There is hardly a book dealing with the subject of bilharziasis which does not show a photograph of a case with distended ascitic abdomen due to the cirrhosis. Such a cirrhosis is held to follow a *mansoni* infestation more often than a *haematobium*.

Another serious result of the disease, stressed by a number of workers from Egypt and South America, is the obstruction that may follow the deposition of ova in the arterioles of the lung. As a result of the obstruction, the tension in the pulmonary artery rises, the right heart enlarges and may eventually fail. In 1938 Shaw and Ghareeb published an important paper pointing out that 2

per cent in their series of bilharzial subjects in Egypt eventually died from right heart failure.

With regard to the appendix, many believe that not only does a chronic, symptomatic appendicitis result, but also not infrequently an acute superimposed inflammation sets in. In other words, the presence of ova in the appendix lowers the resistance in the organ rendering it liable to an acute superadded infection.



FIG. 3. This is an interesting radiograph. In addition to the bladder calcification the ureters can be similarly identified.

It is with urinary tract that so many of the disasters of the disease are coupled. To mention merely the most important, there are: stricture of the ureter often complicated by a hydronephrosis, calculus formation, secondary infection and last but not least, vesical carcinoma.

Having outlined the generally accepted complications of the disease, I shall discuss these in the light of my own experience.

Bilharzial Disease of the Liver. I should like to dwell a short while on the subject of hepatic cirrhosis and bilharziasis. When St. Clair Symmers (1906) of Cairo

found at autopsy ova in the portal tracts of a case with pipe-stem cirrhosis, everyone thought this the answer to the problem of Egyptian splenomegaly. It was soon realized in Egypt that there were many cases of cirrhosis of the liver in whom no ova could be found in the organ itself and it became difficult to explain cirrhosis purely on a bilharzial basis. Nevertheless, not to be beaten it would seem, workers propounded the theory that in those cases of cirrhosis with no ova in the liver it must have been produced by the toxins or poisons set free by the bilharzial worms. No one, however, has demonstrated a toxin with such specific effects on the liver tissue. Experimentally I can find no convincing evidence that either the ova or even the toxins of the disease lead to cirrhosis. This theory seems, for some unknown reason, to have been accepted in other bilharzial countries, notably Japan and South America.

Cirrhosis of the liver is encountered throughout Africa in much the same frequency whether bilharziasis is present or not. For instance in Kampala, Uganda, where the economic and living conditions of the African are similar to those in Southern Rhodesia, but where no bilharziasis is encountered, Muwazi and Trowell (1942) and Davies (1947) find the incidence of cirrhosis of the liver to be about 8 per cent,—much the same frequency as is seen in Southern Rhodesia. In Johannesburg Gillman reports 8 per cent of his African adults show cirrhosis of the liver at autopsy, yet bilharziasis was found in less than 1 per cent of his autopsies. In India and the Far East, too, cirrhosis of the liver occurs with much the same frequency as in Africa.

It would seem much more reasonable to accept that there is some common cause other than the schistosome, for cirrhosis of the liver in Africa, India and the Far East. There is evidence today that the diet of the African, Indian and Japanese is markedly deficient in animal protein and too rich in starchy foods.

Whilst there is no proof that the ova or the so-called toxins can produce a cirrhosis, I do not wish to convey the impression that hepatic lesions are not seen in the liver. Essentially localized ones, due to the ova either *S. haematobium* or *S. mansoni*, are found. (Bilharzioma.) The extent of the fibrosis varies from case to case. The ova are deposited either in the portal tracts or in the parenchyma of the liver, where focal lesions ensue. Thus a bilharzioma results much in the same way as a tuberculoma and gumma arise in tuberculosis and syphilis respectively.

Pulmonary schistosomiasis. The contention that the bilharzial parasite may produce chronic *cor pulmonale* has been mentioned. In view of the very widespread nature of the disease in Southern Rhodesia, it follows that if bilharziasis were as commonly the cause of right heart failure as is stated in the literature, it must be regarded in a very serious light. Clinically I have failed to find a true case. In a series of 400 carefully conducted autopsies on bilharzial subjects I could not demonstrate a single case in which the right heart was hypertrophied and dilated as a result of a pulmonary arteriolar obstruction following upon the deposition of ova. While it is not my intention to deny this possibility I feel justified in stating that it must be a rare cause of death in this part of Africa.

Some workers, notably Turner (1909) in South Africa, have claimed that

schistosomiasis of the lung may invite the onset of a secondary infection such as pneumonia or tuberculosis. Such a postulation at first sight appears to be highly probable. Turner published figures which tended to show that natives on the Rand dying of pulmonary disease were more likely to have ova in the lungs than those who succumbed to non-pulmonary disorders. In a larger series of cases I found the opposite. This claim, in my opinion, has not been satisfactorily proven.

According to my experience, ova (usually *S. haematobium* but sometimes *S. mansoni*) are found in the lungs in about 50 per cent of bilharzial subjects and are purely coincidental to any other pathology in that organ. They are encountered in small numbers, often scattered singly in the parenchyma, appearing to produce little or no tissue reaction around them. So far I have not encountered ova microscopically in such abundance as to be responsible for much functional disturbance.

Appendicitis and Bilharziasis. When Temple Mursell (1912) of the Union, one of the earliest workers in this field, published in the South African Medical Journal the case of an acute perforating appendicitis in a Bantu with bilharzial ova in this organ, the stage was set for the acceptance of yet another serious complication of the disease. The profession readily agreed that the deposition of ova in the appendix reduced its resistance to pyogenic organisms. A few workers, however, began to cast doubt upon this and in 1934 Barsoum of Egypt reported that ova are frequently found in the appendix at autopsy in cases who showed no symptoms during life. He, therefore, came to the conclusion that bilharziasis does not cause appendicitis, acute or chronic. He held that, were bilharziasis the cause of acute appendicitis, one should find it much more commonly in Egypt, where bilharziasis is rife but appendicitis rare.

Lovett-Campbell and Rose (1936) of West Africa, whilst agreeing with Barsoum that bilharzial involvement of the appendix is not complicated by acute appendicitis, maintained that occasionally, when the organ is much fibrosed or thickened, symptoms of chronic appendicitis and appendicular colic may result. They do agree, however, that in the vast majority of cases with bilharziasis no symptoms follow. Nevertheless in Southern Rhodesia the medical profession, as a whole, still regards the presence of ova in the appendix as being the cause of a chronic appendicitis, which is liable to become acute. Indeed, whenever ova are found on section in an excised appendix, they are considered without doubt to be the cause of the symptoms. These views are clearly reflected in a paper published by Rosin in 1943.

In my experience there is little evidence to support the belief that an acute suppurative appendicitis may result from bilharzial involvement of the organ. For instance, using the same argument as Barsoum did, acute appendicitis is rare in the Bantu of Rhodesia in spite of the fact that bilharziasis is so prevalent. The Salisbury Hospital figures show that acute appendicitis is ten times more common in the European than in the African, although the European is affected by schistosomiasis to a much lesser degree than the African. In non-bilharzial regions appendicitis is equally rare in the African. Erasmus (1939) of the Rand

finds appendicitis roughly eight times more frequently in the European than in the Bantu, though in the former bilharziasis was rarely found.

In the Bantu, ova will be found at autopsy in the appendix of every other bilharzial subject infected with the urinary parasite. (*S. mansoni* ova are rarely found in the appendix.) One can expect, therefore, to find ova in this organ in every other case of acute appendicitis in the African. This is actually what I found on studying the acutely inflamed appendices removed at operation in the Bantu. In the European, since the incidence of the disease is so much less than in the African, a smaller percentage of cases with acute appendicitis showed ova. Thus if ova are found in an acutely inflamed appendix, one is justified in assuming that they are merely incidental to the acute infection. Similarly, in spite of the frequent presence of ova in this organ, chronic appendicitis rarely occurs in the African. This is my experience in the Salisbury Native Hospital, where this diagnosis is rarely made. The assumption, therefore, is that in the majority of cases ova do not lead to symptoms. It is only occasionally when the appendix is grossly affected by a massive deposition of ova and resultant fibrosis that symptoms of chronic appendicitis may follow. These findings have an important bearing on clinical practice. It is my opinion that bilharziasis need not be looked upon as a frequent cause of a symptomatic chronic appendicitis, thus only occasionally necessitating removal. More important is that acute appendicitis does not appear to result from a chronically inflamed appendix. Thus, from this aspect, the outlook in bilharzial disease may be viewed more lightly.

The Effects of Bilharzial Disease on the Urinary Tract. The effect of *S. haematobium* on the urinary tract is of great significance, as a number of workers in this field, particularly from Egypt, have attributed serious complications in this part of the body to the disease. These may be grouped as follows:—

- (i) Stricture and stenosis of the ureter with hydronephrosis and its attendant dangers.
- (ii) Calculus formation.
- (iii) Acute superadded infection of the bladder, ureters and pelvis of the kidney.
- (iv) Carcinoma of the bladder.

The Effect on the Ureters. Sooner or later, according to Fairley (1919), Aly Bey Ibrahim (1923), Dew (1923), Byam and Archibald (1923), Campbell Begg (1943), Kirkaldy Willis (1946) and other writers, the passages of the ureters become narrowed, resulting in obstruction to the outflow of urine. Consequently back pressure on the kidneys results, often causing a hydronephrosis on one or both sides. Secondary infection may supervene. Uraemia is liable to occur if both sides are affected. I noticed, at autopsy (1948), that while stricture of the ureter may be encountered, it is much less frequent than actual dilatation by the disease. Instead of a narrowing of the ureter at the site of the disease, there was in the majority of cases dilatation of a varying degree. Hydronephrosis was found to follow dilatation in the absence of stricture. It would appear that the peristaltic function of such a markedly dilated and thickened ureter is impaired and urine is not propelled forwards, thus resulting in increased back pressure.

It is possible too that, when the bladder contracts during micturition, owing to the dilatation which may extend to the orifice itself there may be reflux of urine up the incompetent ureteric orifice with resultant back pressure on the kidneys.

In a series of 250 bilharzial cases at autopsy, about 30 per cent had gross lesions of the ureters. Twenty-seven per cent were due to dilatation alone and only three per cent to stenosis or narrowing of the ureters. Of the 30 per cent with gross lesions, about 15 per cent showed hydronephrosis. Most of these were mild, and in only 1 per cent did the hydronephrosis cause the death of the patient. Thus only a small percentage of bilharzial cases are seriously affected by the hydronephrosis. It takes many years for this complication to be sufficiently advanced to kill the patient. However, in view of the large numbers suffering from the disease, it is by no means negligible.

The African with hydronephrosis is thus able to rear a family before he is eventually carried off by it and the population is thus not much affected. Nevertheless, much sickness and discomfort result from ureteric disease, as it causes abdominal pain, back-ache and other symptoms. It seems doubtful whether anything can be done to check the process once dilatation has set in as fibrosis has already commenced. The disease may be cured by antimony, but not its effects. Repeated dilatation may relieve or perhaps even cure cases of stricture.

The probability is that once a patient has contracted urinary bilharziasis his ureters will be involved as well as the bladder. Serious lesions of the ureter, such as dilatation and stricture, eventually result in about half the cases with ureteric disease. According to my autopsy figures, either dilatation or stricture of the ureter is seen in roughly 1 out of 4 subjects affected with urinary schistosomiasis. Hydronephrosis, however, is found in only a small percentage of these, and fewer still die of it.

Urinary Stone and Bilharziasis. Stone formation due to bilharziasis is held to be common in Egypt. Girges (1934) found it to be present in 8 per cent of patients with the urinary form of the disease. In the African, however, calculus is rarely encountered. Not one case of primary urinary stone was seen in my series of 400 cases with vesical bilharziasis. From these results it would appear that stone formation in the African does not result directly from bilharziasis. Infective phosphatic calculi, however, may occur with the supervention of acute secondary infection such as in a pyonephrosis. I found three such cases of infective calculi in a series of 2,000 autopsies on Africans. On studying the admission figures of the Salisbury Hospital over a period of four years, calculus was found in 0.07 per cent of Africans and in 0.17 per cent of Europeans. It was thus much rarer in the African, although the European is far less often affected by schistosomiasis. Hence it would appear that stone is not usually caused by the disease. In this respect, therefore, the prognosis of bilharziasis is much less serious than it is generally considered.

Secondary Infection of the Urinary Tract. Many references to superadded infections of the urinary tract are found in the literature. The most important of these are by Fairley (1919) and Byam and Archibald (1923). It is difficult to know how often acute secondary infection of the bladder, ureter and pelvis of

the kidneys is caused by bilharzial disease. Acute inflammation of one or more of these parts of the urinary tract was encountered in about 3 per cent of my autopsies. As the vast majority of Africans are found at autopsy to have bilharziasis, it might reasonably be argued that in those relatively few cases with infection the bilharzial disease was incidental, as an African is as liable as is the European to develop acute cystitis or pyelitis, whether or not he has bilharziasis.

Even if cases with acute infection of the urinary tract are held to be due to the disease, there are so few seen clinically and so rarely is the infection the cause of death, that from this point of view bilharziasis need not be considered in a serious light.

It has already been mentioned that hydronephrosis may occur in schistosomiasis. Acute infection, sometimes with urinary stone formation, is liable to develop in such cases. This, however, is not due to the bilharzial disease itself but follows the hydronephrosis.

Vesical Carcinoma and Bilharziasis. One of the gravest complications as yet attributed to bilharziasis is carcinoma of the bladder. It is accepted that cancer of the bladder frequently follows urinary bilharziasis. Ferguson (1911) was the first to point out the high frequency of cancer of the bladder, which he considered due to the irritation of bilharzial ova on its mucosa. Nevertheless he was at a loss to explain the rarity of vesical cancer in women and the fact that the ova did not seem to affect the bowel in the same way. His theory was supported by other writers such as Chenhall (1915), Fairley (1919 and 1930), Makar (1932) and Kirkaldy Willis (1946). A few others, however, such as Girges (1934), although agreeing that bladder cancer was caused by the irritative action of ova, nevertheless reported this complication as rare and not so prevalent as found by some workers. Smith and Elmes (1934) from Nigeria wondered how it was that carcinoma of the bladder is not more common in the African in view of the high incidence of urinary bilharziasis in him.

In Southern Rhodesia I have found bladder cancer to be relatively uncommon, although not rare. In a series of 2,000 autopsies, it was found in 5 cases (0.25 per cent), as was carcinoma of the stomach. The latter is regarded by most workers in the African field, such as Berman (1936), as being one of the more uncommon growths in the Bantu. This would, therefore, apply to cancer of the bladder. It is far less frequent than primary carcinoma of the liver, which was found in 21 cases in the same series. It cannot be argued that because bilharzial ova are found in the bladder in an endemic region such as Mashonaland that they were the cause of the cancer, since ova can be expected in practically every bladder in the African in this area. On the other hand, the incidence at autopsy of bladder cancer in areas where schistosomiasis is not so prevalent as in Southern Rhodesia is much the same. In 1,900 autopsies, Strachan (1934) found 5 cases of carcinoma of the bladder, two of these in bilharzial subjects. A more useful comparison is the figure for vesical carcinoma given to me in a personal communication by Davies (1948) of Kampala, where bilharziasis is rarely seen. He found 3 cases of malignancy of the bladder in a series of 2,000 autopsies.

Another point which would tend to indicate that carcinoma of the bladder is

not related to bilharzial disease is that on studying the admission figures for Salisbury Hospital over a four-year period, I found the incidence of bladder cancer to be 0.09 per cent for Europeans and 0.05 per cent for Africans. Thus, although bilharziasis is much more frequent in the African than in the European, bladder cancer is much less often seen in him. This is borne out, too, by Strachan's experience in the Transvaal, where he finds at autopsy that bladder cancer is twice as frequent in the European as in the Native.

In view of these findings, therefore, it would appear that bilharziasis either does not ordinarily lead to carcinoma of the bladder or only rarely. If this is the case, the prognosis of the disease becomes much less grave. More statistical evidence is clearly required to determine this point.

Effect of Bilharzial Disease on Other Organs. It is not easy to estimate the damage to organs such as the gall bladder, pancreas, spleen and stomach by bilharzial disease. Their involvement would appear to cause little danger to life. On the other hand, when the Fallopian tube is affected there is the liability to an ectopic pregnancy.

When portions of the brain, gall bladder, pancreas, stomach, uterus and spleen are digested in caustic potash, ova may not infrequently be found. These are usually distributed throughout the organs in greater or smaller numbers. Although bilharzial cholecystitis, pancreatitis, and gastritis may be present from the pathological point of view, it is difficult to prove clinically that symptoms attributable to the involvement of a certain organ are in fact caused by ova in that particular organ. Dyspepsia, for example, may result from bilharzial disease of the liver, pancreas or gall bladder. It may, on the other hand, be reflex from the appendix or from some other distantly related viscus. It is more likely to be due to involvement of the liver, since ova are found in this organ in approximately half of all bilharzial cases, whereas they are seen far less commonly in the pancreas, gall bladder or stomach. I have noticed, too, that the liver is usually involved at the same time, when these other organs are affected.

The involvement of the central nervous system by bilharzial disease is of significance, since a paraplegia may result from the heavy deposition of ova in a localized area of the spinal cord. The exact frequency of such an eventuality is difficult to estimate, but I have encountered such a case. I have not, as yet, however, seen bilharzial tumors or granulomata of the brain, which are described as producing symptoms similar to those of a glioma and are recorded in Asiatic schistosomiasis. According to my autopsy figures, ova are deposited in the brain in 5 per cent of bilharzial cases and still less frequently in the spinal cord. It is claimed that such scattered deposits of ova, in contradistinction to the granuloma, may produce epilepsy, memory disorders and lack of concentration. Although such disturbances are known to be relieved by antimony therapy there is no satisfactory proof that these disorders are caused by bilharzial involvement of the brain.

In conclusion, in view of the specifically localized effects of the disease, it may be inferred that bilharziasis is not so serious as is claimed in the literature. If it is confirmed that multilobular cirrhosis of the liver and vesical cancer are not due

to the disease, it must be viewed much less gravely. If, as has been shown, stone and secondary infection rarely complicate the disease, in this respect too the prognosis is less serious.

According to my findings, the deposition of ova in the appendix does not appear to invite secondary infection with a resultant acute appendicitis, and symptoms of chronic appendicitis are produced but rarely.

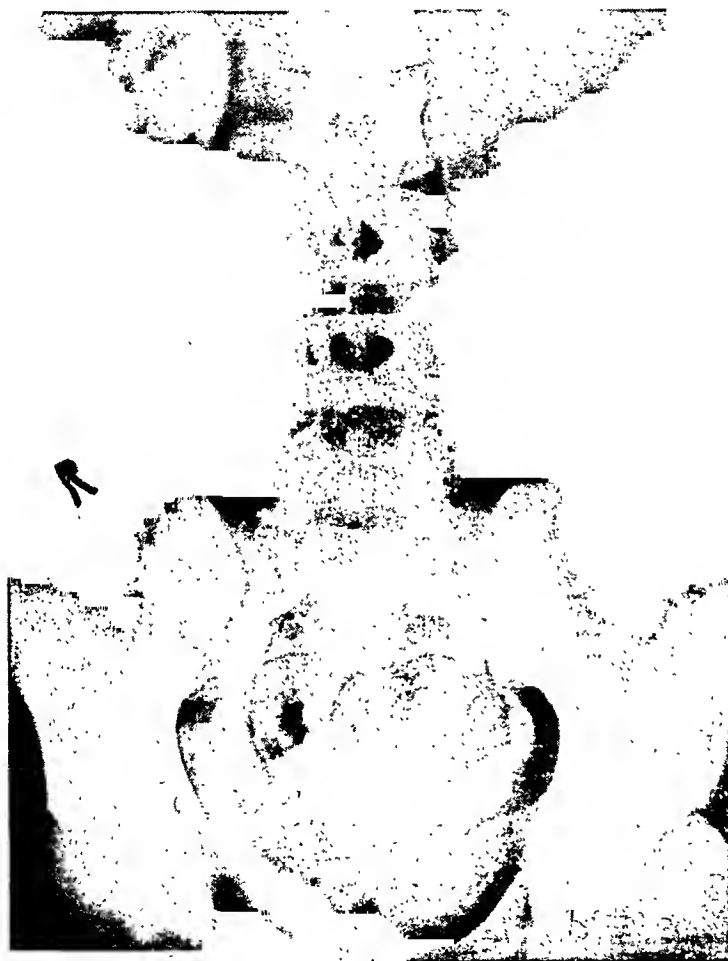


FIG. 4. A PYELOGRAM SHOWING THE CHARACTERISTIC SAUSAGE-SHAPED DILATATION OF THE LOWER PORTIONS OF THE URETERS PRODUCED BY THE DISEASE

Further, the claims made in Egypt that 2 per cent of bilharzial subjects are liable to develop right heart failure are not confirmed by my experience. On the other hand I find that the true gravity of the disease must be attributed to its effect on the ureters, where the deposition of ova in the walls of these tubes may result in dilatation and less frequently in stricture (Fig. 4). In some of the cases hydronephrosis may follow either the dilatation or stricture. Death from uraemia, pyelonephritis and pyonephrosis may occasionally occur as a result of the hydronephrosis. According to my autopsy figures, death occurs in only about 1 per

cent of bilharzial cases. Although the mortality rate is not high, it is of significance when it is recalled that the vast majority of the African population are sufferers from the disease. However, if the views expressed in this paper are confirmed by other workers, it is obvious that many of the existing ideas concerning certain of the grave effects on the different viscera of the body attributed to the disease may have to be reconsidered.

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THE TREATMENT OF TRICHOCEPHALIASIS WITH LECHE DE HIGUERON¹

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Intestinal infection with *Trichocephalus trichiurus* (whipworm) usually does not produce appreciable symptoms. However, it is too frequently considered to be a completely harmless condition. There are many reports (1-9) of severe symptoms and even death which compel the admission of the occasional seriousness of trichocephaliasis. These clinical complications which have been described include secondary anemia, nervous disturbances and such gastro-intestinal disorders as appendicitis, peritonitis and dysentery. Because such symptoms do occur, it is well to look upon this infection with less indifference and to consider critically the therapeutic agents available.

All of the various anthelmintic drugs in common use have been employed at one time or another in the treatment of trichocephaliasis, and all of them have been reported to be rather ineffectual with the exception of one, the sap or latex obtained from certain species of wild fig trees (*Ficus* spp.) in Central America. Customarily, this latex is referred to as "leche de higuerón". Since Bajon (10), 150 years ago, first scientifically described its use in treatment of whipworms among natives of Cayenne, several dozen accounts have appeared in the medical literature. These many reports have been well summarized by Thomen (11), and contain estimates of therapeutic efficiency varying widely from 16.6 per cent (12) to 85.4 per cent (13). Because of such discrepancy in results, there has been controversy for many years about the actual worth of leche de higuerón, and the present investigation was undertaken in an attempt to clarify this disagreement.

Robbins (14) in 1930 isolated the active principle from crude leche de higuerón, and described it as a proteolytic enzyme of the tryptic type. He further showed (15) that there was a marked seasonal variation in the amount of enzyme present per unit of sap, the concentration being lowest in the early summer; this probably accounts for some of the difference in previously reported therapeutic efficiency. It was also demonstrated by others that the enzyme lost its potency through fermentation with the passage of time, even with the addition of sodium benzoate as a preservative (*i.e.*, as in such proprietary products as "Higueronia"). Best results were usually described when the crude substance was immediately placed in refrigeration and used early, although the highest therapeutic rating of all reports (85.4 per cent) was in a careful study (11) in which a preparation of the sap of *F. laurifolia* was used which had been kept cool in dark bottles for almost a year. It was further shown that not all species of wild fig trees could be used as a source, but that *Ficus glabrata*, *F. carica* L., *F. crassiuscula*, *F. laurifolia* and several others were adequate sources.

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MATERIAL AND METHODS

This report covers 127 cases of trichocephaliasis observed at Gorgas Hospital, Panama Canal Zone, treated with fresh leche de higuerón and studied by means of pre-treatment and post-treatment egg counts. The individuals treated varied in age from one to forty years. Two of them were of the white race, the remainder were of negro or mestizo stock. There was no difference in therapeutic results in the several age and race groups.

The leche de higuerón was obtained from either of two wild fig trees, *F. crassiuscula* or *F. glabrata*, by trained personnel of the Canal Zone Experiment Gardens. It was taken from the trees at high tide, because it was the impression of Experiment Garden personnel that at such times it is more plentiful and runs more freely. Collections were made during the late wet-season months of October, November and December, in much the same way that native rubber is collected. The leche was immediately placed in clean dark brown bottles and put in refrigeration. In-vitro tests on live *Trichocephalus* and *Ascaris* worms showed the substance to be rapidly and highly lethal. It was used in the treatment of the patients with trichocephaliasis within two weeks of the time of collection.

The regimen which was followed in the administration of the leche de higuerón and in the collections of stools was as follows:

1. Stools were collected for two days before giving the leche, and three to five egg counts were performed on each of these stools after thorough mixing to ensure even sampling.

2. A light supper was allowed the night before treatment, and breakfast was delayed on the morning of treatment.

3. At 6:00 A.M., 60.0 cc. of the fresh leche was administered by mouth, diluted with 120.0 cc. of milk.

4. If spontaneous catharsis did not occur within two hours of treatment, 60.0 cc. of 50 per cent magnesium sulfate solution was administered at 8:00 A.M. Breakfast was then allowed.

5. No stools were examined for eggs on the day of treatment, and counts done on the first day after treatment were discarded as unreliable. Multiple egg counts were done on thoroughly mixed stools collected on the third, seventh and eighth days thereafter.

The Egg-Count Method: Eggs were counted by the direct smear method devised by Beaver (16). This method is based on the photoelectric measurement of turbidity of the smear. By means of a frosted glass standard furnished by Beaver, our instrument was calibrated to give smears bearing one-three hundredths cc. of formed feces.

Postulating the number of trichocephalus adult worms from the number of eggs in the feces is admittedly inaccurate. This is in part due to the worms' distalward location, which in some cases may cause uneven distribution of eggs in the fecal mass. More important is the lack of reliable data on the actual rate of egg production. Different people have made very different estimates; some as low as 1,000 eggs per day, others up to 50,000 eggs per female per day. Probably the best published estimate is that of Manalang (17) who on the basis of a small series of autopsy cases determined that there were approximately 150 eggs per

female worm per gram of formed stool, and that there were equal numbers of male and female worms present.

TABLE I

Percentage reduction in Trichocephalus eggs following administration of leche de higueron

INTENSITY OF INFECTION	CASE	BEFORE TREATMENT			AFTER TREATMENT			PER CENT REDUCTION IN EGGS
		No. Stools	No. Counts	Average Eggs/cc.	No. Stools	No. Counts	Average Eggs/cc.	
Light (less than 2,500 eggs per cc. of formed stool)	1	2	10	1,980	2	10	990	50
	2	3	15	630	2	10	360	43
	3	2	10	1,800	2	10	0	100
	4	2	10	1,400	4	20	0	100
	5	3	15	240	4	20	0	100
	6	2	10	300	2	10	60	80
	7	1	5	1,800	2	10	540	70
	8	2	10	1,125	2	10	600	47
	9	2	10	90	1	8	0	100
	10	2	10	150	1	8	0	100
	11	2	10	2,280	2	10	1,050	54
	12	1	8	600	2	10	450	25
	13	2	10	600	3	9	180	70
	14	2	6	1,140	2	6	1,200	0
Average of light cases.....								67.1
Moderate (Between 2,500 and 25,000 eggs per cc. of formed stool)	15	2	8	3,259	4	12	630	81
	16	3	12	13,350	3	12	5,850	56
	17	2	10	12,825	2	8	3,900	70
	18	2	8	4,620	4	12	1,600	65
	19	2	6	3,750	1	6	1,800	52
	20	2	10	4,050	2	10	3,150	22
	21	2	8	12,000	2	6	4,800	52
Average of moderate cases.....								56.9
Heavy (More than 25,000 eggs per cc. of formed stool)	22	2	10	380,400	2	10	50,700	87
	23	1	6	81,000	3	15	48,600	40
	24	3	12	42,030	2	8	25,650	39
	25	1	6	99,900	3	15	13,350	87
	26	3	12	26,540	3	12	17,000	36
	27	2	10	45,450	2	10	14,250	69
Average of heavy cases.....								59.7
Average of all cases.....								62.8

THERAPEUTIC RESULTS

Table I presents the results of administration of leche de higuerón to patients with varying degrees of infection with *Trichocephalus*. The per cent reduction of eggs before and after treatment is similar in the groups, and the average reduction of eggs in all cases is 62.8 per cent. It is of interest that only in cases

with very low infection was there an apparent cure at any time. These 5 cases (see Table I) had so few eggs before treatment that they might easily have passed for negative stools had not a number of examinations been made.

Four of the patients harbored *Ascaris* as well as whipworm, and *Ascaris* egg counts were also done before and after leche de higuierón was administered to these individuals. Approximately three-fourths of the *Ascaris* worm burden was removed by this medication, as judged by the egg counts. None of the patients, however, was cured. The actual total egg count of the four patients before treatment was 451,879 per cubic centimeter. After treatment, the total egg count was 127,575 per cubic centimeter.

Approximately one-half of the individuals who received leche de higuierón had abdominal discomfort, nausea and/or vomiting. In all instances when vomiting was provoked, it occurred after a period of 1½ to 2 hours following the administration of the drug, and was considered not to have influenced the anthelmintic effect of the leche because the regimen called for catharsis after two hours anyway. None of the symptoms observed in patients of this series was of an alarming nature.

DISCUSSION

Although most infections with *T. trichiurus* are apparently asymptomatic, the number of those with serious symptoms is of sufficient magnitude to make an adequate treatment regimen highly desirable. Helminthologists, pediatricians and other interested individuals have been taught that leche de higuierón is an excellent substance with which to treat trichocephaliasis, if only it can be given immediately to avoid fermentation or loss of potency. We desired to test the validity of this assumption by administering the leche under essentially ideal conditions, obtaining it locally, placing it in refrigeration and using it as quickly as possible. As the results indicate, approximately ⅔ of the worm burden was removed by a single dose of the substance. This is a substantial reduction and would suggest that in areas where the leche is easily available it would be worthwhile to treat and perhaps re-treat selected cases. It does not give outstanding results, however, and is certainly not the final answer to the need for a therapeutic agent even in areas where the wild fig trees grow. If even under favorable circumstances, only 60 per cent of the worms can be removed by a dose, it would suggest that search for a more efficient anthelmintic for this infection should be made.

SUMMARY

1. Fresh leche de higuierón, collected and administered to patients under what was considered to be ideal conditions, removed approximately 60 per cent of *Trichocephalus* eggs.

2. This drug caused minor gastro-intestinal symptoms in approximately 50 per cent of patients.

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THE IMPORTANCE OF SANITATION IN MUNICIPAL FLY CONTROL¹

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Prior to the discovery of the insecticidal value of DDT, sanitation had long been recognized as one of the principal basic fly control measures, and municipal laws and regulations governing the maintenance of horses, cattle, and other domestic livestock within city limits had long since removed the most prolific sources of fly breeding in most cities. The work reported herein was initiated in 1948 to obtain data on the relative importance of present-day sources of fly breeding in urban areas, which might be useful in guiding community-wide fly control efforts.

Early advocates of fly control soon recognized the undesirable aspects of attempting to control adult flies without adequate attention being directed toward the prevention of fly breeding. Dr. L. O. Howard (1911) aptly stated the case:

"... the truest and simplest way of attacking the fly problem is to prevent them from breeding, by the treatment or abolition of all places in which they can breed. To permit them to breed undisturbed and in countless numbers, and to devote all our energy to the problem of keeping them out of our dwellings, or to destroying them after they have once entered in spite of all obstacles seems the wrong way to go about it."

The discovery of the almost incredible powers of DDT as an insecticide, however, brought about many changes in attitudes toward fly control and fly control measures. The success which accompanied its initial use for fly control seemed to vindicate the claims of its most enthusiastic endorsers, and highly responsible workers in the field of insect control began to visualize possible area eradication of house flies and certain other insects with DDT and other new insecticides (Lyle, 1947).

The immediate response to these developments was an awakened public interest in fly control *by chemical means*. County-wide, and in some instances state-wide, fly control programs have been conducted in many sections, practically all based on the use of DDT or one of the other newly developed insecticides. Although most competent scientific workers cautioned against any let-down in sanitation practices, the public's enthusiasm for DDT and other of the newer insecticides has undoubtedly resulted in decreased attention to basic sanitation practices. Andrews and Simmons (1948) attributed some of the failure of DDT to obtain the expected degree of fly control to poor sanitation, which resulted in high levels of fly production.

Poor sanitation, however, is not the only cause of the recent decrease in the effectiveness of DDT in some localities. Missiroli (1948) has reported a strain

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of house fly in Italy that is naturally resistant to DDT. Strains of house flies resistant to DDT, benzene hexachloride, chlordan, and other insecticides have been reared in the laboratory (Blickle *et al.*, 1948; Lindquist and Wilson, 1948; Wilson and Gahan, 1948). In some of these strains, the resistance is more or less specific to DDT or its very close relatives; in others, the flies appear to be resistant to a variety of insecticides. Other workers have definitely established the fact that resistant strains of flies have appeared in the field in areas where DDT has been used repeatedly for the past few years (Fay and Buckner, in press; Barber and Schmitt, 1948). This development of insect resistance to DDT and other new powerful insecticides is not contrary to past experience. Quayle (1943) mentioned seven other insect pests which had developed resistance to old, established insecticides such as lime-sulfur, arsenicals, cyanide, phenothiazine, and tartar emetic.

The recent tendency for the public to place more dependence on chemical fly control and perhaps less on sanitation, together with the prospects of increasingly frequent appearances of insecticide-resistant strains of flies, seemed to make this an appropriate time to re-emphasize the importance of sanitation in municipal fly control. The work discussed in this paper was undertaken with the objective in mind of developing information on present fly breeding sources in urban areas in order that municipal fly control efforts, especially those involving improved sanitation practices, might be directed toward those objectives which would be expected to yield the highest returns in accomplished fly control. These studies were conducted in Savannah, Georgia, a city of approximately 130,000 population. While the city as a whole may not be typical of all urban areas, it contains environmental areas which are common to practically all American cities; efforts were made to select these typical environmental areas for making the investigations.

SURVEY OF FLY BREEDING SOURCES IN URBAN AREAS

Surveys were made of such potential house fly and blow fly breeding sources as the city garbage dump, garbage cans, privies, stables, abattoirs, grocery stores, creameries, feed stores, poultry houses, and fertilizer plants. In addition, over-all premises inspections of residential areas were made, in which a search was conducted for any possible fly breeding sources such as dog feces small animal, carcasses, grass cuttings, chicken yards, and so on. The number of premises surveyed in each environmental area varied from 101 to 150, which was sufficient to establish a uniform trend of fly breeding in the various types of breeding material in each area.

Considering the various fly breeding sources in the city from an over-all viewpoint, it was concluded that the city garbage dump was the most important single source of flies in the city. The dump covered an area of approximately 15 acres, was located just outside the city limits, and produced enormous numbers of many species of flies. Dispersal tests, described later, indicated that blow flies could and did move freely from the dump back into the city proper.

The second principal source of house flies and blow flies in the city was the

garbage can. Approximately 60 per cent of all garbage containers throughout the city were found to be actively producing flies in the soft accumulation of "garbage sludge" in the bottoms of the cans, or in the soil immediately under unserviceable cans. The term "garbage sludge" refers to the soft garbage which accumulates in the bottom of dirty garbage cans and is not removed by normal garbage collection, but remains constantly in the cans. The percentage of garbage containers found to be producing flies in this manner at the time of the survey was 69 per cent in the best residential area, 62 per cent in the middle-class apartment district, 56 per cent in the tenement section, and 61 per cent in the business district. Species of flies found breeding in or immediately under garbage cans included *Musca domestica*, *Muscina stabulans*, *M. assimilis*, *Fannia pusio*, *Hermetia illucens*, *Stomoxys calcitrans*, *Phaenicia pallescens*, *P. cluvia*, *P. sericata*, *P. eximia*, *Callitroga macellaria*, *Euxesta nitidiventris*, *Plecticus testaceus*, *Dendrophonia querceti*, *Drosophila melanogaster*, *Sarcophaga bullata*, *S. haemorrhoidalis*, *S. lambens*, *S. sarracenoides*, *S. ventricosa*, several unidentified species of *Sarcophaga*, *Limonia schwarzi*, *L. domestica*, and unidentified specimens of Psychodidae. No strenuous effort was made to collect specimens breeding in the garbage cans, and the above list undoubtedly can be expanded for this locality. Also, specimens not common to this area likely will be found breeding in garbage cans in other localities.

In residential areas, dog feces ranked next to the garbage cans in neighborhood fly production. The percentage of inspected premises which were positive for fly breeding in dog excrement was 41 per cent in the best residential area, 20 per cent in the middle-class sections, and 7 per cent in the tenement district. A total of 123 specimens of naturally infested dog feces was collected and the flies allowed to emerge from them. The number of flies produced from a single specimen varied from one to 588, with an average of 144 flies per specimen for the 123 collected. Burial tests were conducted in which three naturally infested dog specimens were buried in each of a series of holes varying in depth from 3 to 18 inches. The soil was refilled loosely in half of the holes and repacked as tightly as possible in the others by trampling with the feet and pounding with a shovel. The number of flies infesting the stools was not known and probably varied considerably, so that no estimate can be made as to percentage of emergence. Some emergence occurred from every depth and type of refill tested, however, and the number of flies emerging from some of the 18-inch packed refill tests actually exceeded the number of those emerging from other less severe tests. It was concluded therefore that the burial of fly-infested dog excrement was ineffectual. Picking up the feces and placing it in the garbage can might be a practical method of disposal of this source of fly breeding in residential areas. The bulk of the flies found breeding naturally in dog feces was made up of *Sarcophaga laticetosa*, *S. stimulans* and *S. ventricosa*. In addition to these three, other species of flies found developing in dog feces included *Musca domestica*, *Callitroga macellaria*, *Sarcophaga sueta*, *S. floridensis*, *S. haemorrhoidalis*, *S. cingarus*, undetermined species of *Fannia* and Larvaevoridae.

Privy surveys indicated that over 90 per cent of the privies supported active

fly breeding at the time of the examination. Most of the privies in this city are the open surface type rather than the pit type. Flooding of the privies or other temporary local factors were responsible for the lack of breeding in those few privies in which it was not observed. It was concluded that 100 per cent of the privies in Savannah are involved in fly production at one time or another. There are only slightly over 400 privies in Savannah, however, and this relatively small number reduces the significance of this source of flies. No special effort was made to determine the density or the entire variety of species of flies breeding in privies, but recognized species included *Hermetia illucens*, *Telmatoscopus albipunctatus*, *Ophyra leucostoma*, *Fannia pusio*, *Sarcophaga galeata*, *S. haemorrhoidalis*, *S. latisetosa*, *S. ventricosa*, and other undetermined *Sarcophaga* species. *Hermetia illucens* appeared to be encountered most frequently, although *Ophyra leucostoma*, Psychodidae, and *Sarcophaga* were also numerous.

Abattoirs, poultry houses, and stables ranked high as individual producers of house flies and blow flies, but the small number of such establishments reduced their importance in the over-all fly production in the city.

No fly breeding of significance was found in or around grocery stores, feed stores, chicken yards, creameries, or fertilizer plants, although some of these establishments are highly attractive to the adult flies. The garbage containers were the only important source of fly breeding around these establishments. No canneries or vegetable packing establishments are located in Savannah.

INVESTIGATIONS OF THE DISPERSAL OF A YELLOW-EYED MUTANT STRAIN OF *CALLITROGA MACELLARIA* IN URBAN AREAS

During May and June 1948, studies were made of the dispersal from the city dump of a yellow-eyed mutant strain of the common blow fly, *Callitroga macellaria*, isolated and developed in this laboratory (Mead and Fay, in press). Figure 1 shows diagrammatically the location of the city dump, point of fly release, and location of the traps in the city. The dump area is just outside the northeast corner of the city limits, about 3 miles from the center of the business district; however, during the war residential areas were extended to within less than one-half mile of the dump area. When these investigations were in progress, garbage and refuse of all types were unloaded on the surface of the dump area, the combustible material was burned, and the remaining material was bulldozed over the edge of the dump, which was being used to fill in a low, marshy area. Enormous numbers of flies were produced on the dump, where there was a vast quantity of material attractive to adult flies. The purpose of the release tests was to determine if the flies would readily leave such an attractive area as the garbage dump and move back into the city.

Laboratory-reared pupae were exposed in a protected receptacle on the dump area so that the emerging adults were free to leave at will. To check on the absence of the yellow-eyed strain in nature, the traps were operated 12 days prior to release of the flies, without the recovery of any yellow-eyed specimens. The traps were located at one-half mile intervals along five lines radiating from south-southwest to due west of the dump area for a distance of 3 miles. In addition,

one trap was placed on the dump area about 200 yards north of the point of release and one trap a quarter of a mile west of the release point. The release point was at the southern edge of the most active portion of the dump area, so that the trap on the dump area was in direct line of flight between the release point and the most active part of the dump.

From May 10 to June 11, approximately 57,000 yellow-eyed flies were released on the dump. The first yellow-eyed specimen was recovered on May 14 in a trap located in a residential area 2 miles from the point of release. The traps were operated until June 21 and during the entire trapping period a total of 21 yellow-eyed flies was recovered in 10 of the 32 traps operated. Seventeen different recoveries were made, some of which represented duplicate recoveries

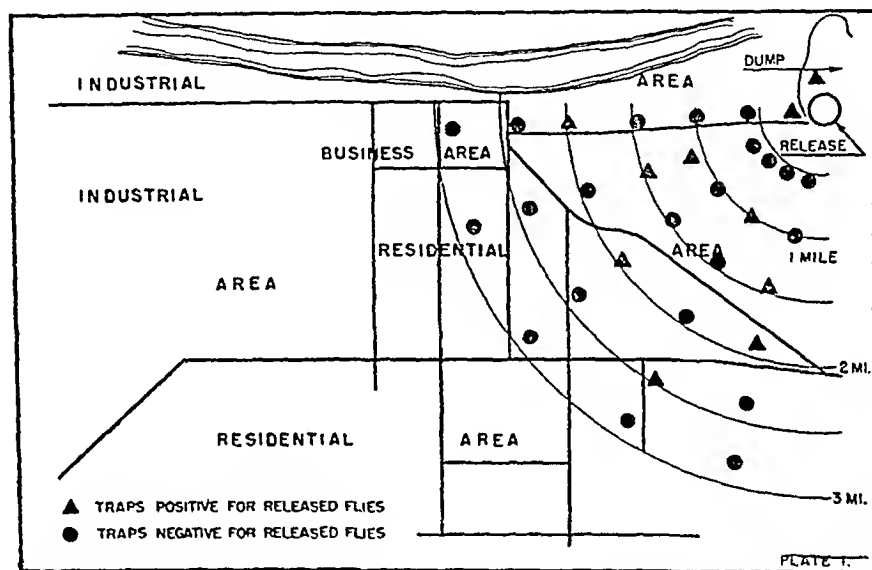


FIG. 1. DISPERSAL OF YELLOW-EYED MUTANT STRAIN OF *CALLITROGA MACELLARIA* FROM CITY GARBAGE DUMP

SAVANNAH, GEORGIA MAY-JUNE 1948

in the same trap. Recoveries had been made in seven different traps ranging from $\frac{1}{4}$ to $2\frac{1}{2}$ miles in or toward the city from the release point, before any yellow-eyed flies were taken on the dump area in the trap only 200 yards distant from the release point. Recoveries were made on four different dates in the trap located $\frac{1}{4}$ mile west of the release point toward the city, and on two different dates in each of three traps located 1, $1\frac{1}{2}$, and 2 miles into the city from the release point. This compared to only two recoveries in the trap on the dump only 200 yards from the point of release. The greatest distance of recovery was $2\frac{1}{2}$ miles into the city from the release point.

In October, a second release test was made to check the speed of dispersal of the yellow-eyed flies within the city proper. Approximately 50,000 adult yellow-eyed flies were released at the approximate geographical center of the residential area of the city (Figure 2). The weather was sunny and cool, follow-

ing 10 days of cool rainy weather. The temperature was 64°F. at the time of the release and reached a maximum of only 72°F. during the day. The flies appeared very sluggish and many were observed lying or resting on the ground at the release point several minutes after release. Twenty-eight traps were located in concentric circles at distances of approximately $\frac{1}{4}$, $\frac{1}{2}$, 1, and $1\frac{1}{2}$ miles from the point of release. In addition, one trap was placed 2 miles to the northeast and one 2 miles to the northwest from the release point. The traps were operated 16 days before the release, with no yellow-eyed flies being recovered in them. Pick-up of the trap catches began at 2:00 p.m. on October 5, following the release of 10:15 a.m. that morning. Pickup began at the traps nearest the release point. Recoveries of yellow-eyed flies were made in two traps picked up between 2:00

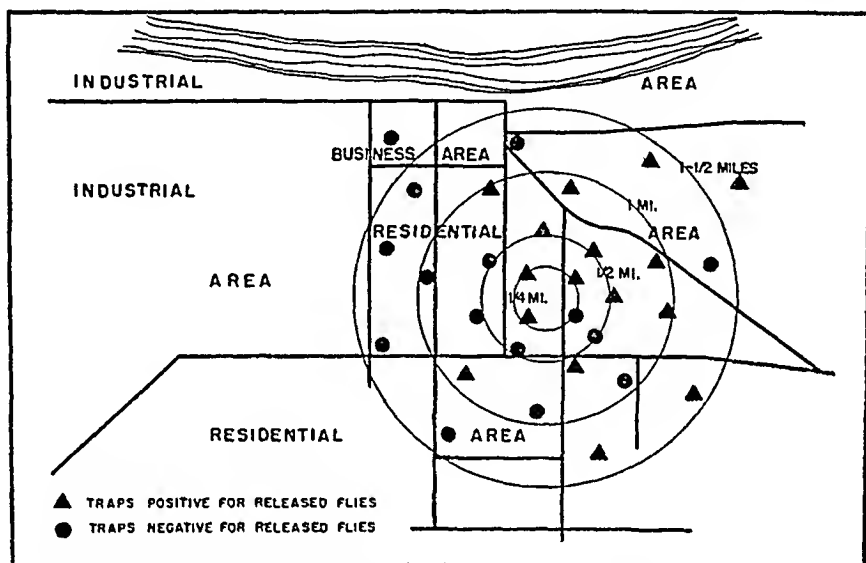


FIG. 2. DISPERSAL OF YELLOW-EYED MUTANT STRAIN OF *CALLITROGA MACELLARIA* IN AN URBAN AREA

SAVANNAH, GEORGIA OCT. 5-13, 1948

and 2:15 p.m. at distances of $\frac{1}{4}$ mile to the northeast and north-northwest of the release point; in a trap 1 mile west of the release point which was picked up at 5:30 p.m.; and in a trap $1\frac{1}{2}$ miles south of the release point which was picked up at 6:00 p.m. In addition, the trap collectors observed yellow-eyed flies on a trap 1 mile northeast of the point of release at 4:30 p.m. and on a trap 1 mile distant east-southeast at 5:00 p.m. Prevailing winds on that date were from the north and northwest. On the day following release, when the prevailing wind was from the northeast, yellow-eyed flies were taken in 4 traps ranging from $\frac{1}{4}$ to $1\frac{1}{2}$ miles to the northeast and north-northwest of the release point. On the second day with the wind from the southeast, they were recovered in 7 traps ranging from $\frac{1}{4}$ to $1\frac{1}{2}$ miles to the north-northwest, east, and south of the release point. During the 9 days following the release of the flies, a total of 39 different recoveries was made in 16 different traps, a total of 69 specimens being recovered. Most of the

recoveries were made in traps located in the eastern half of the trapping area. One recovery was made in the trap placed 2 miles to the northeast of the release point, one of the two traps located that distance away.

These release tests indicate clearly that this species of blow fly will move freely from a city dump several miles back into the city proper. They also showed that this fly will disperse from a central focal point over a distance up to $1\frac{1}{2}$ miles, and in all directions, within seven hours. The weather conditions prevailing at the time of the second release were not favorable for maximum fly activity, and more rapid dispersal may occur under more favorable conditions. Thus, these tests indicate that the fly problem in a city is one of city-wide importance, as heavy fly production in one neighborhood would supply flies to other parts of the city.

DISCUSSION

The relative importance which any of the fly breeding sources discussed above may assume in any given urban area will vary with local conditions. Garbage collection and disposal methods and facilities are very important factors in municipal fly control and should receive first consideration in any city where fly control is contemplated. Privies are important in areas where the proportion of privies is greater than in Savannah. This is especially true because of the greater significance which may be attached to flies developing in privies. However, in the absence of more adequate knowledge as to the precise role of the various species of flies in disease transmission, or even as to what species are so involved, it is difficult to establish a definite priority on the desirability of control of any few species. The interrelationship of the factors involved is indicated by the fact that in the limited collections of specimens and observations made in this study, it has been noted that several species breed interchangeably in garbage cans, dog feces, privies, stables, and waste products of industrial food establishments.

Dog feces are apt to be of considerable significance in most urban residential areas, as a source of both fly attraction and fly breeding. As a result of limited, preliminary studies, Wolff *et al.* (1948) reported the isolation from dogs of 16 types of *Salmonella* which were serologically, biochemically, and morphologically similar to those frequently infecting man, birds, and other animals. Flies breeding in or feeding upon dog feces might well be considered as potential sources of *Salmonella* infection for man.

Stables, poultry houses, and abattoirs may be more important in smaller communities where their number and distribution are proportionately greater than in Savannah. In areas where food canneries are operated, the waste products from such establishments may overshadow all others in fly production.

In planning a municipal fly control program, the important fly-producing sources in each city should be determined by thorough surveys made by competent observers in advance of actual control measures, so that operations may be intelligently directed against the various fly breeding sources in the order of their relative importance.

One of the most striking impressions gained in conducting the work described herein, was that the ultimate answer to the municipal fly control problem is simple sanitation, rather than the continuous use of expensive chemical control except as auxiliary measures, or in special or emergency situations. Other workers (Watt and Lindsay, 1948) also have concluded that fly control by insecticidal methods is a temporary expedient and have stressed the need for the elimination of man-made fly breeding places. There may be a marginal level of fly production in urban areas below which it is not economically feasible to attempt to reduce it by further sanitation efforts where insecticides may be used to drive down that level without excessive cost. However, this has not yet been demonstrated since most communities where chemical fly control is practiced could greatly reduce their pretreatment levels of fly production through improved sanitation.

For example, fly breeding in city garbage dump areas could be eliminated by the adoption of any one of several suitable sanitary garbage disposal methods. Picking up dog feces at frequent intervals and placing them in the garbage can appears to be a practical means of controlling fly breeding from this source—a job for the individual dog owner. The enactment and enforcement of municipal regulations requiring the citizenry to provide adequate, serviceable garbage containers, and to keep them clean, would eliminate the garbage can as a significant source of fly production. An ordinance requiring the wrapping of garbage, as is now required in a number of large cities, would accomplish much toward preventing the accumulation of “garbage sludge” in the cans. Privies could be, and should be, eliminated from most cities where they are still found.

These procedures for the sanitary collection and disposal of garbage, and the elimination of privies would greatly reduce the fly incidence in residential areas. Operators of abattoirs, stables, poultry houses, canneries and other establishments whose by-products or waste products are responsible for large-scale fly breeding in urban areas should be required to devise some means of disposing of such material in a sanitary manner as an essential and legitimate part of their operating procedure. This is commonly done in the case of oil refineries, chemical plants, and paper manufacturers whose waste products constitute a menace to fish and wild life or are a public nuisance.

During the work discussed above, no important source of fly breeding was found in the city which would not lend itself to correction by simple sanitation. Such sanitation practices in addition to reducing, if not eliminating, the fly control problem, would provide additional benefits in the form of a reduced incidence of rats, roaches, and other vermin which thrive on the same types of material that constitute fly breeding sources. Consequently, in approaching the problem of municipal fly control from a long-range standpoint, good sanitation practices should be considered as one of the most important, effective, and economical fly control measures, and every effort should be put forth to bring its advantage to the attention of the public. This is especially true in view of the increasing accumulation of evidence that flies are developing resistance to more than one of the newer chemicals formerly considered as panaceas of the fly control problem.

SUMMARY

In the city of Savannah, Georgia, which has an estimated population of 130,000 people, surveys were conducted to determine the present sources of house flies and blow flies in the city.

Fly dispersal tests were conducted using laboratory-reared flies of a yellow-eyed mutant strain of the common blow fly *Callitroga macellaria*. These tests indicated that this species of fly would move readily from the city dump back into the city proper. Tests conducted under rather adverse weather conditions with respect to fly activity showed that this species would travel in all directions from the point of release in the city and for distances up to 1½ miles within seven hours.

Results of the fly release tests and surveys of fly breeding sources indicated that the city garbage dump, located approximately 3 miles from the heart of the business district, was the principal source of flies in the city. Garbage cans and dog feces were the only important sources of fly breeding found in residential areas. Fly breeding in the "garbage sludge" accumulated in the bottom of garbage containers, or in the soil under inadequate or unserviceable containers, was found to occur in approximately 60 per cent of all containers examined. The percentage of containers involved in active fly production at the time of survey in the various typical urban areas was 69 per cent in the best residential area, 62 per cent in the middle-class section, 56 per cent in the tenement district, and 61 per cent in the business district. The percentage of premises found positive for fly breeding in dog stools was 41 per cent in the best residential area, 20 per cent in the middle-class section, and 7 per cent in the tenement district.

All privies in the city probably produce flies, but the number of privies in the city is small. Abattoirs, poultry houses, and stables are high producers of flies, but such establishments are not numerous. No significant fly breeding was found at fertilizer plants, grocery stores, creameries, feed stores, or neighborhood chicken yards.

The relative importance of the various fly breeding sources in cities will vary with local conditions, and each city should be the subject of a thorough survey by competent observers in advance of attempting municipal fly control.

No fly breeding sources in Savannah were observed which could not be reduced greatly or eliminated entirely by improved sanitation practices.

Considering the added benefits of partial control of rats, roaches, and other vermin of public health importance which would accompany fly control by improved sanitation, and the appearance in widely separated areas of strains of flies resistant to DDT and certain other insecticides, it seems appropriate to re-emphasize and strongly recommend sanitation as an important, effective, and economical means of municipal fly control.

ACKNOWLEDGMENTS

Grateful acknowledgment is made to Dr. Clair A. Henderson, City-County Health Officer of Savannah, Georgia, whose excellent cooperation made it pos-

able to conduct the work reported on herein. Special thanks are also due to Dr. Harold R. Dodge, Dr. R. Edward Bellamy, and Mr. Willis Mathis for their assistance in fly identifications, and to Mr. Kenneth M. Mead and Dr. R. W. Fay for the production of the yellow-eyed flies used in the release tests. Mr. Bernard O. Smith performed most of the routine trap collections, and Messrs. H. M. Berman and Robert E. Barker assisted in examination of the collections for the presence of the yellow-eyed flies.

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LETTER TO EDITOR

Tse-Tse Fly and Trypanosomiasis
Permanent Inter-African
Bureau
Léopoldville (Belgian Congo)
P.O. Box 557
Telegrams: Tsetse Léopoldville

The Editor,
American Journal of Tropical Medicine,
Tallahassee, Florida, U. S. A.

Dear Sir,

This Bureau would be grateful if you would kindly call, through the intermediary of your publication, on the doctors and biologists who are interested in African Trypanosomiasis and would like to receive our publications and information on the matter, to contact us at the following address.

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With a view to facilitating matters for us, we should appreciate if our correspondents would add to their communications, summaries which will be published in our bulletin.

We are, Dear Sir,

Sincerely yours
For the Directors of the B.P.I.T.T.
DR. J. CECCALDI AND DR. G. NEUJEAN

BOOKS RECEIVED

Note: Books received for editorial consideration will be intermittently listed. This acknowledgement must be regarded as an adequate expression of appreciation for the courtesy of the author or publisher. Selections will be made for review in the interest of our readers.

- ROBERTO LEVI CASTILLO, Entomologo-Sanitario (Culicidologo). *Atlas de las Anofelinos Sudamericanos*. Pp. 207, figs. 354 (on plates) (dibujos de Walter Vivar A.) wrap. Guayaquil, Ecuador. Tip. de la Sociedad Filantrópica del Guayas. 1949. \$10.00 (U. S.).
- E. H. CLUVER, Director. *Annual Report for the Year 1948: The South African Institute for Medical Research*. pp. 63, Johannesburg.
- MARCO ANTONIO CABRERA Y J. ROMEO DE LEON. *Historia del Primer Caso Clinico de Leishmaniasis Visceral (Kala Azar) Descubierto en Guatemala*. Pp. 36, figs. 13, wrap. Pub. del Inst. de Investigaciones Cientificas, No. 2. Guatemala, C. A., 1949.
- HAROLD WELLINGTON JONES, NORMAND L. HOERR AND ARTHUR OSOL, Editors. *New Gould Medical Dictionary*, 1st Edition. 1294 pp., 252 illus. (129 in color), Fabricoid. Philadelphia, The Blakiston Company, 1949. Prices: \$8.50, \$10.75, \$13.50.
- LEONARD A. SCHERLE, Surgeon General. *Annual Report of the Public Health Service, Extract Report Federal Security Agency, 1948*. pp. xii, 255-453. wrap. Washington, Government Printing Office, 1949.
- ELEANOR T. CAVERLY. *How to be Healthy in Hot Climates*. Pp. xii, 275. Clo. New York, Thomas Y. Crowell Company, 1949. \$3.00.
- PROF. DR. THEODOR WAGNER-JAUREGG. *Therapeutische Chemie. Arznei- und Desinfektionsmittel zur Bekämpfung von Infektionskrankheiten*. Pp. 272. wrap. Bern, Verlag Hans Huber, 1949. Sfr. 35.50. Distributed by Grune & Stratton, 381 Fourth Ave., New York City.

BOOK REVIEWS

CARLOS MONGE. *Acclimatization in the Andes*. Pp. vii plus 111 plus 6 reference plus 8 index. Institute of Andean Biology, University of San Marcos, Lima, Peru, 1948. John Hopkins Press, Baltimore, Maryland.

It is the author's thesis that life at high altitudes deserves a new biology concerned with beings adapted to reduced atmospheric oxygen tension. This field of biology illustrates the principle that the reactions of living organisms are related to the duration as well as to the intensity of imposed stresses. There is every extreme in response ranging from the vomiting newcomer to the hard-working, healthy resident.

The research conducted by the author and his associates has been in two directions. He has looked back to the history of the inhabitants of the uplands of the Andes. This region, lying above 10,000 feet altitude, has been the home of millions of people for hundreds of years. "Acclimatization in the Andes" is based on his historical research. This is revealed by the chapter headings, *Evidence of Climatic Aggression, Fertility and Acclimatization, Individual Acclimatization, Racial Acclimatization, and Altitude and Military Operations*.

Dr. Monge has looked not only to the past. As a clinical investigator and physiologist, he has inquired into the mechanism of acclimatization. Not much of that research is included in this book, but his expert knowledge of the biology of man adapted to high altitudes has enabled him to interpret historical records in a fascinating manner.

In the foreword, Isaiah Bowman points out the importance of this work to the economist, the sociologist, the engineer, and the philosopher. It will also be of interest to the physician, including specialists in the field of tropical medicine. Some of these specialists will want to make use of the research opportunities afforded by the Institute of Andean Biology directed by the author. This institute now has laboratories at Lima (Medical School of the University of San Marcos), Huancayo (11,000 feet), and Morococha (14,800 feet). These laboratories deserve generous support; with sufficient encouragement the Institute might be enabled to establish another unit in its chain of laboratories,—a jungle laboratory east of the Cordilleras. In the author's closing words:

"The key to success is additional support for truly scientific work of much broader scope, and facilities for beyond those provided up to the present time."

D. B. DILL

HAROLD WELLINGTON JONES, NORMAND L. HOERR AND ARTHUR OSOL, Editors. *New Gould Medical Dictionary*, First Edition. pp. xxviii and 1294. Figs. 252 (129 in color) grouped in 45 plates. Flexible fabricoid, fore-edge index. The Blakiston Company, Philadelphia, 1949. \$8.50.

This completely new unabridged medical dictionary has been compiled by a board of editors with the assistance of more than one hundred contributors, and is designed to be a comprehensive lexicon for all branches of medicine and allied sciences, including medical physics and chemistry, dentistry, pharmacy, nursing, veterinary medicine, biology, botany and medico-legal terms. Pronunciation is indicated by a system of phonetic respelling with syllabification. The volume terminates with a series of twenty-two tables requiring 136 pages.

The circumstance that the work is a new entity, compiled by a large group of specialists in the respective fields covered, should guarantee that within its scope, the work is in the highest degree authoritative.

MARK F. BOYD

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